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CONTENTS

Page

OFFICE OF THE DIRECTOR

Summary Statement 3

PROGRAMS OF THE OFFICE OF THE DIRECTOR

INTERNATIONAL PROGRAMS

Office of the Assistant to the Director for International Programs, Summary Statement. 9

FACILITIES ENGINEERING BRANCH

Facilities Engineering Branch, Summary Statement 17

HEALTH AND SAFETY BRANCH

Health and Safety Branch, Summary Statement. 25

LIBRARY AND INFORMATION SERVICES BRANCH

Library and Information Services Branch, Summary Statement. 33

DIVISION OF INTRAMURAL RESEARCH

Office of the Scientific Director, Division of Intramural Research Summary Statement. 41

COMPARATIVE MEDICINE BRANCH

Summary Statement. 47
Individual Project Reports 50

LABORATORY OF CELLULAR AND MOLECULAR PHARMACOLOGY

Summary Statement. 55
Individual Project Reports 58

LABORATORY OF GENETICS

Summary Statement. 65
Individual Project Reports 67

LABORATORY OF MOLECULAR BIOPHYSICS

Summary Statement. 75
Individual Project Reports 78

LABORATORY OF MOLECULAR CARCINOGENESIS

Summary Statement. 107
Individual Project Reports 110

LABORATORY OF MOLECULAR GENETICS

Summary Statement. 117
Individual Project Reports 121

LABORATORY OF MOLECULAR AND INTEGRATIVE NEUROSCIENCE

Summary Statement. 151
Individual Project Reports 154

LABORATORY OF PULMONARY PATHOBIOLOGY

Summary Statement. 175
Individual Project Reports 178

LABORATORY OF REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

Summary Statement. 185
Individual Project Reports 188

DIVISION OF BIOMETRY AND RISK ASSESSMENT

Office of the Director, Division of Biometry and Risk Asessment

Summary Statement 199
Individual Project Reports 203

COMPUTER TECHNOLOGY BRANCH

Summary Statement 207

EPIDEMIOLOGY BRANCH

Summary Statement 213
Individual Project Reports 216

LABORATORY OF BIOCHEMICAL RISK ANALYSIS

Summary Statement 227
Individual Project Reports 231

STATISTICS AND BIOMATHEMATICS BRANCH

Summary Statement 241
Individual Project Reports 244

DIVISION OF TOXICOLOGY RESEARCH AND TESTING

Office of the Director, Toxicology Research and Testing Program

Summary Statement 251

CARCINOGENESIS AND TOXICOLOGY EVALUATION BRANCH

Summary Statement 259
Individual Project Reports 265

CELLULAR AND GENETIC TOXICOLOGY BRANCH

Summary Statement 277
Individual Project Reports 281

CHEMICAL PATHOLOGY BRANCH

Summary Statement 305
Individual Project Reports 308

SYSTEMIC TOXICOLOGY BRANCH

Summary Statement 323
Individual Project Reports 326

INDEX TO INDIVIDUAL PROJECT REPORTS v

INDEX TO INDIVIDUAL PROJECT REPORTS

DIVISION OF INTRAMURAL RESEARCH

COMPARATIVE MEDICINE BRANCH

Project Number	Title	Page
Z01 ES 22103-05 CMB	Natural History of Mouse Hepatitis Virus	50
Z01 ES 22107-03 CMB	Natural History of PVM	51

LABORATORY OF CELLULAR AND MOLECULAR PHARMACOLOGY

Z01 ES 80001-16 LCMP	Microsomal Mixed-Function Oxidase System: Structure and Function	58
Z01 ES 80007-16 LCMP	Conjugation and Oxidation Pathways for Xenobiotic Metabolism	59
Z01 ES 80031-12 LCMP	Role of Altered Membrane Function in Xenobiotic Toxicity	60
Z01 ES 80042-02 LCMP	Calcium Regulation and Signal Transduction Mechanisms	61
Z01 ES 80043-01 LCMP	Ion Channel Modulation by Signal Transduction Systems	62

LABORATORY OF GENETICS

Z01 ES 60099-09 LG	Organization-regulation of Mammalian Lactate Dehydrogenase Genes	67
Z01 ES 61019-08 LG	Collaborative Protein Sequencing and Peptide Synthesis	68
Z01 ES 61032-05 LG	Structure-function of Mammalian Lactate Dehydrogenase Isozymes	69
Z01 ES 65021-16-LG	Investigation of Germinal Mutation Induction in Mice	70
Z01 ES 65033-05 LG	In Vivo Mammalian Mutagenesis	71

Project Number	Title	Page
	LABORATORY OF MOLECULAR BIOPHYSICS	
Z01 ES 10004-09 LMB	NMR Studies of the Mechanisms of Cell Injury	78
Z01 ES 30003-17 LMB	Development of Analytical Methodology	79
Z01 ES 50046-10 LMB	Mechanisms of Chemically Induced Photosensitivity	80
Z01 ES 50080-06 LMB	Environmental Health Applications of Mass Spectrometry	81
Z01 ES 50082-05 LMB	Studies on Tumor Promoters and Antipromoters	82
Z01 ES 50087-02 LMB	Mechanisms of Singlet Oxygen-Dependent Photosensitivity	83
Z01 ES 50088-02 LMB	Relationship of Free Radicals to Halocarbon-Induced Toxicity in the Liver	84
Z01 ES 50089-02 LMB	The Reaction of Free Radical Metabolites with DNA	85
Z01 ES 50090-02 LMB	Porphyrin Ion Radical Metabolites and Their Reactions	86
Z01 ES 50091-02 LMB	Phenyl Radical Formation by Oxyhemoglobin from Phenylhydrazine In Vivo	87
Z01 ES 50092-02 LMB	The Mechanisms of Reduction of Nitroheterocyclic Drugs	88
Z01 ES 50093-02 LMB	NMR Studies of the Metabolism of Leishmania Braziliensis	89
Z01 ES 50094-02 LMB	NMR Studies of Dihydrofolate Reductase	91
Z01 ES 50095-02 LMB	In Vivo F-19 NMR Studies of the Metabolism of Fluorinated Anesthetics	92
Z01 ES 50096-02 LMB	Changes in Tissue Non-Cyclic Phosphodiesters Produced by Toxins	93
Z01 ES 50097-02 LMB	Development and Application of an OTLC-MS Interface	94
Z01 ES 50098-02 LMB	Development of FAB/MS and FAB/MS-MS for Environmental Health Sciences	95
Z01 ES 50099-02 LMB	Application of Thermospray LC-MS to Structure Elucidation of Biomolecules	96

Project Number	Title	Page
Z01 ES 50100-02 LMB	Structure Elucidation of Carcinogen-Nucleoside Adducts	97
Z01 ES 50101-02 LMB	Identification of Tetrachlorodibenzofuran Metabolites	98
Z01 ES 50102-02 LMB	Mass Spectral Analysis of the Benzo[a]Pyrene-DNA Reaction Product	99
Z01 ES 50103-02 LMB	GC-MS Analysis of PCDF Blood Levels in Children Exposed In Vitro	100
Z01 ES 50104-02 LMB	In Vivo NMR Studies of Cellular Magnesium	101
Z01 ES 50105-01 LMB	Analysis of Airway Epithelium Prostaglandins	102
Z01 ES 80008-14 LMB	Biosynthesis of Prostaglandins, Hydroxy-Fatty Acids and Leukotrienes	103
Z01 ES 80035-12 LMB	Cooxidation of Xenobiotics by the Prostaglandin Synthetase	104

LABORATORY OF MOLECULAR CARCINOGENENIS

Project Number	Title	Page
Z01 ES 23000-01 LMC	Genetic Events in Heptacarcinogenesis of B6C3F1 Mice	113
Z01 ES 25001-11 LMC	Role of Mutagenesis in Carcinogenesis	110
Z01 ES 25029-04 LMC	Mechanisms of Neoplastic Transformation by Viral and Cellular Oncogenes	111
Z01 ES 25031-02 LMC	Role of Tumor Suppressor Genes and Oncogenes in Chemical Carcinogenesis	112

LABORATORY OF MOLECULAR GENETICS

Project Number	Title	Page
Z01 ES 60147-05 LMG	Molecular Mechanisms of SOS-Mutagenesis in Escherichia coli	121
Z01 ES 60148-05 LMG	Error-Prone Repair in Bacteriophage T4	122
Z01 ES 61022-07 LMG	The Population Genetics of Transposable Elements	123
Z01 ES 61024-06 LMG	Genetic and Molecular Analysis of Suppressor-of-Sable Function in Drosophila	124

Project Number	Title	Page
Z01 ES 61030-05 LMG	Molecular Analysis of the *Om* mutation in Drosophila *ananassae*	125
Z01 ES 61037-04 LMG	Mechanism of DNA Replication in Eucaryotes: Yeast as a Model System	126
Z01 ES 61039-04 LMG	Mechanims of DNA Recombination and Repair in the Yeast Saccharomyces cerevisiae	127
Z01 ES 61041-02 LMG	Molecular Genetic Variation in Natural Populations	128
Z01 ES 61042-02 LMG	Gene Expression in Early Development of Drosophila	129
Z01 ES 65034-04 LMG	The Specificity of Spontaneous and Induced Mutation	130
Z01 ES 65036-04 LMG	Gene Organization and Regulation in *D. Melanogaster*	131
Z01 ES 65037-04 LMG	Transposon - Mediated Chromosome Instabilities in Drosophila	132
Z01 ES 65038-03 LMG	Molecular Mechanisms of Mutagenesis by Animal Cell DNA Polymerases	133
Z01 ES 65041-02 LMG	DNA Repair in Mammalian Cells	134
Z01 ES 65042-02 LMG	Role of Gene *uvsW* in Error-Prone Repair by Bacteriophage T4	135
Z01 ES 65043-02 LMG	Role of Gene *uvsX* in Error-Prone Repair by Bacteriophage T4	136
Z01 ES 65044-02 LMG	Replication Reapir in Bacteriophage T4	137
Z01 ES 65045-02 LMG	Bacteriophage T4 *rI* Mutations	138
Z01 ES 65046-02 LMG	Accuracy of DNA Replication *in vitro*	139
Z01 ES 65047-02 LMG	Fidelity of Retroviral Reverse Transcriptases	140
Z01 ES 65048-02 LMG	Engineering DNA Polymerases to Probe Mutational Mechanisms	141
Z01 ES 65049-02 LMG	Mechanisms of Mutagenesis With Yeast Replication and Repair Proteins	142
Z01 ES 65050-02 LMG	Molecular Analysis of Deletion Mutations in Chinese Hamster Ovary Cells	143

Project Number	Title	Page
Z01 ES 65051-02 LMG	Molecular Analysis of Point Mutations in Chinese Hamster Ovary Cells	144
Z01 ES 65052-02 LMG	Use of Retroviral Vectors in the Analysis of Mutations in Mammalian Cells	145
Z01 ES 65053-01 LMG	DNA Sequence Characteriztion of Bacteriophage T4 rII Mutations	146
Z01 ES 65054-01 LMG	Invariant Per-Genome Mutation Rates	147

LABORATORY OF MOLECULAR AND INTERGRATIVE NEUROSCIENCE

Project Number	Title	Page
Z01 ES 70090-05 LMIN	Neuroendocrine and Neurochemical Regulation of Gonadal Function	154
Z01 ES 70092-05 LMIN	Cellular and Molecular Mechanisms Mediating Peptide Hormone Action	155
Z01 ES 70096-04 LMIN	Regulation of Pulsatile Gonadotropin Secretion	156
Z01 ES 90033-06 LMIN	Milk Bombesin and Kinins	157
Z01 ES 90034-05 LMIN	Rabbit Stomach Peptide (Physalaemin-like-material [PHLIM]) in Mammalian Tissue	158
Z01 ES 90039-05 LMIN	Modulation of Brain Opiods and Tachykinins by Neurotransmitter Systems	159
Z01 ES 90042-03 LMIN	Models of Neurodegenerative Processes Involving Cognitive and Motor Dysfunction	160
Z01 ES 90043-03 LMIN	Role of Zinc in Synaptic Transmission in the Hippocampal Formation	161
Z01 ES 90044-03 LMIN	Modulation of Neuronal Function by Neuropeptides and Steroid Hormones	162
Z01 ES 90045-03 LMIN	Studies on the Relationship between Opioid Peptides and Seizures	163
Z01 ES 90047-02 LMIN	Neuroregulatory Aspects of Neuromedin B	164
Z01 ES 90048-03 LMIN	Physiology and Pharmacology of Neuropeptides	165
Z01 ES 90049-02 LMIN	Molecular Regulation of the Hormonal Output from Adrenomedullary Chromaffin Cells	166
Z01 ES 90050-02 LMIN	Roles of Opioid Peptides in the Regulation of Hippocampal Excitability	167

Project Number	Title	Page
Z01 ES 90051-02 LMIN	Brainstem and Spinal Cord Modulation of Neurological Motor Dysfunction	168
Z01 ES 90052-01 LMIN	Compensation and Recovery of Function in the Central Nervous System	169
Z01 ES 90053-01 LMIN	Neuropeptides: Molecular Mechanism of Action	170
Z01 ES 90054-01 LMIN	Regulation of Biosynthesis, Processing and Secretion of Neuropeptides	171

LABORATORY OF PULMONARY PATHOBIOLOGY

Z01 ES 25020-06 LPP	Regulation of the Pulmonary Surfactant System and its Modification by Toxic Agents	178
Z01 ES 25021-05 LPP	Regulation of Differentiation of Tracheobronchial Epithelial Cells	179
Z01 ES 25023-05 LPP	Cellular and Molecular Mechanisms of Progression of Transformed (RTE) Cells	180
Z01 ES 25027-05 LPP	Identification and Characterization of Materials Secreted by Pulmonary Clara Cells	181
Z01 ES 25030-02 LPP	Cellular and Biochemical Mechanisms of Particle-Induced Lung Disease	182

LABORATORY OF REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

Z01 ES 70010-11 LRDT	Study of Normal and Abnormal Embryonic Development	188
Z01 ES 70060-15 LRDT	Developmental Biology/Toxicology of Estrogenic Environmental Chemicals	189
Z01 ES 70062-01 LRDT	The Role of Growth Factors and Inhibitors in Estrogen-induced Uterine Growth	190
Z01 ES 70065-12 LRDT	Chemical-Receptor Interactions in Reproduction and Hormonal Toxicity	191
Z01 ES 70067-05 LRDT	Molecular Mechanism of Steroid Hormone in Sex Organ Development	192
Z0! ES 70076-04 LRDT	Germ Cell-Specific Molecules of Spermatozoa	193

Project Number	Title	Page
Z01 ES 70077-02 LRDT	The Expression of Heat-Shock Genes in Mouse Spermatogenic Cells	194
Z01 ES 70078-05 LRDT	Characterization of Stage-Specific Surface Antigens During Mouse Spermatogenesis	195
Z01 ES 80040-05 LRDT	Developmental Pharmacogenetics of Microsomal Steroid Hydroxylases	196

DIVISION OF BIOMETRY AND RISK ASSESSMENT

OFFICE OF THE DIRECTOR, DBRA

Project Number	Title	Page
Z01 ES 43010-02 DBRA	Macromolecular Modeling and Carcinogenesis	203

EPIDEMIOLOGY BRANCH

Project Number	Title	Page
Z01 ES 43002-12 EB	Human Exposure to Halogenated Aromatic Compounds	216
Z01 ES 43004-10 EB	Environmental Exposures and Chronic Renal and Other Diseases	217
Z01 ES 43008-08 EB	Biochemical and Cellular Environmental Epidemiology	218
Z01 ES 44003-11 EB	Epidemiologic Study of Reproductive Outcomes and Environmental Exposures	219
Z01 ES 46002-04 EB	Environmental Exposures and Cancer Risk	220
Z01 ES 47001-02 EB	Exposure to Radon-222 and Cancer Mortality	221
Z01 ES 47002-02 EB	Biological Effects of Plant Estrogens in Postmenopausal Women	222
Z01 ES 48003-02 EB	The Etiologic Significance of Oncogene Activation in Human Bladder Cancer	223
Z01 ES 48004-02 EB	Health Effects of Passive Exposure to Cigarette Smoke	224

LABORATORY OF BIOCHEMICAL RISK ANAYLSIS

Project Number	Title	Page
Z01 ES 21024-07 LBRA	Drug Metabolizing Enzymes in Animal Models and Human Tissue	231

Project Number	Title	Page
Z01 ES 35005-09 LBRA	Mechanisms of Carcinogen-Induced DNA Damage and Cell Transformation	232
Z01 ES 43009-05 LBRA	Role of Kidney & Nutritional Factors in Metabolism of Toxic & Essential Metals	233
Z01 ES 46003-04 LBRA	Lymphocyte Markers for Evaluating Exposure and Biologically-effective Dose	234
Z01 ES 46004-04 LBRA	Receptor Interactions and Liver Tumor Promotion	235
Z01 ES 46005-04 LBRA	Oncogene Activation and Expression in Rodent Tumors	236
Z01 ES 48005-01 LBRA	Biochemical Mechanisms Related to Risk Factors of Mammary Carcinogenesis	237
Z01 ES 70069-06 LBRA	DNA Adducts in Human Lymphocytes and Hormone-Dependent Cancers	238

STATISTICS AND BIOMATHEMATICS BRANCH

Project Number	Title	Page
Z01 ES 40004-11 SBB	Statistical Methods in Epidemiology	244
Z01 ES 44002-12 SBB	Mathematical Modeling of Molecular Phenomena	247
Z01 ES 45001-08 SBB	Experimental Design and Data Analysis Methodology for Animal Experiments	245
Z01 ES 48001-01 SBB	Statistical Analysis of Data from Genotoxicity Experiments	248
Z01 ES 48002-01 SBB	Statistical Models in Toxicology and Biochemistry	246

DIVISION OF TOXICOLOGY RESEARCH AND TESTING

CARCINOGENESIS AND TOXICOLOGY EVALUATION BRANCH

Project Number	Title	Page
Z01 ES 21050-05 CTEB	Evaluation of Microencapsulation As a Means to Administer Chemicals in Feed	265
Z01 ES 21076-05 CTEB	Cellular Biochemistry Studies on Chemicals Selected for Evaluation by NTP	266

Project Number	Title	Page
Z01 ES 21078-05 CTEB	Bioavailability and Toxicity Studies of Microencapsulated Chemicals	267
Z01 ES 21079-05 CTEB	Mechanism of Di(2-ethylhexyl)phthalate Hepatotoxicity	268
Z01 ES 21095-02 CTEB	Development of In Vitro Propagated F344/N Mononuclear Cell Lines	269
Z01 ES 21096-02 CTEB	Identification and Isolation of c-fms Photooncogene From F344/N Rat Leukemia	270
Z01 ES 21097-02 CTEB	Evaluation of Chemical Myelotoxicity Using an In Vivo Leukemia Transplant Model	271
Z01 ES 21102-02 CTEB	Dermal Absorption of Diethanolamine and Triethanolamine in Rats and Mice	272
Z01 ES 21108-01 CTEB	Biochemical Toxicology of a Mixture of 25 Groundwater Contaminants	273

CELLULAR AND GENETIC TOXICOLOGY BRANCH

Project Number	Title	Page
Z01 ES 21012-07 CGTB	Organ and Species Specificity of Chemical Carcinogenesis	281
Z01 ES 21013-07 CGTB	Molecular Analysis of Gene Toxic/Carcinogenic Events in Mammalian Cells	282
Z01 ES 21016-07 CGTB	Enzymes Involved in DNA Repair and Meiosis	283
Z01 ES 21032-04 CGTB	Development of Peroxidase Oxidation Systems in Mutation Assays	284
Z01 ES 21035-04 CGTB	Structural Analysis of Meiotic Chromosome Behavior in Yeast and the Mouse	285
Z01 ES 21039-04 CGTB	Genetic Control of Sister Chromatid Exchange in Yeast	286
Z01 ES 21045-06 CGTB	Analysis of SPO11, a Gene Required for the Early Events of Meiosis in Yeast	287
Z01 ES 21048-05 CGTB	Development of a Molecular System to Study Mutagenesis in Yeast	288
Z01 ES 21049-06 CGTB	DNA Synthesis and Metabolism During Meiosis	289
Z01 ES 21051-05 CGTB	Cytogenetic Analysis of Mutagen-Sensitive Mutants	290

Project Number	Title	Page
Z01 ES 21052-06 CGTB	Metabolism of Xenobiotics to Mutagens Using Non-hepatic Microsomal Enzyme Systems	291
Z01 ES 21053-05 CGTB	Genetic Control of Mutation in Drosophila	292
Z01 ES 21054-05 CGTB	DNA Damage and Repair in Centromeres of Yeast	293
Z01 ES 21091-03 CGTB	Effects of DNA Lesions on Untargeted DNA Metabolic Events	294
Z01 ES 21094-02 CGTB	Mutagenesis by Free Radicals From Bisulfite and Thiols	295
Z01 ES 21101-02 CGTB	Mutant Selection Due to Differential Toxicity	296
Z01 ES 21103-02 CGTB	Evaluation of Genetic Toxicity Test Results	297
Z01 ES 21106-01 CGTB	In situ Protocols for Mammalian Cell Mutagenesis Assays	298
Z01 ES 21107-01 CGTB	Mutagenicity Studies of Hydrogen Peroxide and Hydrogen Peroxide Generating Systems	299
Z01 ES 60102-10 CGTB	Testing of Chemicals of Interest in Salmonella	300
Z01 ES 60122-09 CGTB	Molecular Mechanisms of DNA Repair in Yeast and Their Role in Meiosis	301

CHEMICAL PATHOLOGY BRANCH

Project Number	Title	Page
Z01 ES 21074-04 CPB	Effects of Glycol Ethers on Bone Marrow Parameters	313
Z01 ES 21080-04 CPB	Nuclear Magnetic Resonance Imaging Facility	314
Z01 ES 21082-03 CPB	Residual Marrow Effect from Ethylene Glycol Monomethyl Ether (EGMME) Exposure	315
Z01 ES 21083-03 CPB	Myelotoxicity Induced in Female B6C3F1 Mice by Methyl Isocyanate Inhalation Exposure	316
Z01 ES 21098-02 CPB	Adverse Effects of Lindane in B6C3F1 Mice	317
Z01 ES 21099-02 CPB	Hematopoietic Effects in Female B6C3F1 Mice Exposed to Arsine Gas	318
Z01 ES 21100-02 CPB	Residual Hematopoietic Effect of Ochratoxin A (OCT A) in Mice Exposed to Irradiation	319

Project Number	Title	Page
Z01 ES 21111-01 CPB	Stability and Tissue Reaction of an Implantable Identification Device	308
Z01 ES 21112-01 CPB	Growth Patterns of F344 Rats Fed NIH-07 and NTP-88 Diets	309
Z01 ES 21113-01 CPB	Myelotoxicity in Mice caused by Drinking Mixture of Groundwater Contaminants	310
Z01 ES 21114-01 CPB	Pancreatic Nodule Transplantation	311
Z01 ES 21115-01 CPB	Effects of d-Limonene on a-2U-Globulin in Rat Kidney	312

SYSTEMIC TOXICOLOGY BRANCH

Z01 ES 21003-08 STB	Disposition of Halogenated Dibenzofurans	326
Z01 ES 21004-08 STB	Senescent Changes in Metabolism	327
Z01 ES 21009-07 STB	Reproductive Effects in Males Exposed to Environmental Chemicals	328
Z01 ES 21031-04 STB	Computer Simulation of Inhalation Exposures	329
Z01 ES 21033-04 STB	Disposition of Xenobiotics	330
Z01 ES 21034-04 STB	Evolution of Metallothionein-like Proteins in Non-Mammalian Species	331
Z01 ES 21038-06 STB	Chemical Metabolism and Disposition	332
Z01 ES 21046-05 STB	Postnatal Toxicology	333
Z01 ES 21070-05 STB	TCDD Teratogenicity: Modulation in Mixtures	334
Z01 ES 21075-05 STB	Xenobiotic Metabolism	335
Z01 ES 21084-03 STB	Association of Chemically Induced Forestomach Cell Proliferation & Carcinogenesis	336
Z01 ES 21086-03 STB	2-Butoxyethanol Hematotoxicity: Effects of Age and Metabolism	337
Z01 ES 21089-02 STB	Mechanism of Action of Testicular Toxicants	338
Z01 ES 21090-03 STB	Mechanisms of Arsine Gas and Gallium Arsenide Toxicity	339
Z01 ES 21093-02 STB	Mechanism of Dioxin Toxicity	340

Project Number	Title	Page
Z01 ES 21105-01 STB	Heat Shock Proteins in Tsticular Somatic Cells	341
Z01 ES 21109-01 STB	Mechanisms of 2-Butoxyethanol Induced Hematotoxicity	342
Z01 ES 21110-01 STB	Developmental Immunotoxicity Studies of Inbred Mice	343
Z01 ES 30044-12 STB	Toxicology of Environmental Chemicals	344
Z01 ES 30106-14 STB	The Effects of Environmental Pollutants on the Immune System	345
Z01 ES 70200-14 STB	Molecular Mechanisms for Regulating for Intracellular Bioavailability of Metals	346

OFFICE OF THE DIRECTOR

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The National Institute of Environmental Health Sciences, while pursuing and developing its scientific programs in all four of its major organizational Divisions, has also attained major accomplishments in fulfilling its mandate under the Superfund Amendments and Reauthorization Act (SARA) of 1986. This Act provided that NIEHS administrate the Superfund Basic Research Program, to provide basic biomedical research on health effects related to Superfund programs and concerns. In addition, NIEHS has been assigned the role of funding training programs for waste dump workers and others who handle or supervise those who handle hazardous substances, under the NIEHS-funded Superfund Worker Training Grants.

In meeting the Superfund Basic Research Program committment, the Department of Health and Human Services assembled an exceptional group of distinguished scientists, public health officials, industry and labor representatives, and other concerned members of the public to serve on the Department of Health and Human Services Advisory Council on Hazardous Substances Research and Training, which met for its second annual session in July 1988, at the NIEHS facility in Research Triangle Park, N.C. In turn, a Subcommittee on Research Coordination was appointed to return to the Council with specific recommendations for discussion.

Presently, grants under the NIEHS Superfund Basic Research Program have been made to the Department of Environmental Health at the University of Washington, for the study of "Effect-Related Monitors of Toxic Exposure"; to the Massachusetts Institute of Technology to look at the chemicals present in humans and to determine which of these cause genetic changes; to University of California at Davis to study the concentration of materials at hazardous waste sites and to relate this to human exposures; and to University of California at Berkeley and San Francisco, to study new techniques for characterizing and quantifying chemical exposures. Moderate budget increases for this Program, should they be allocated, would augment these present efforts; whereas, more substantial increases might enable NIEHS to make grants to new institutitons.

Eleven NIEHS-Funded Superfund Worker Training Grants were made in September 1987. Awardees include five union groups, five university coalitions, and one local fire department. These groups projected in their applications that they will collectively train over 200,000 workers before the program's end.

The new initiatives under Superfund have only underscored the importance of the knowledge base continuously enhanced by the programs of the four major Divisions within NIEHS. A number of major publications by our scientists highlight a few research advances which act as an introduction to those covered in complete detail in the body of this Report.

Scientists in the Division of Biometry and Risk Assessment published in The New England Journal of Medicine the results of a nine-year study of early pregnancy loss. Data from this study indicated that 22 percent of pregnancies were lost before they were clinically detected, and that these losses typically occurred in fertile women who later successfully delivered healthy infants. This cruicial baseline study can now be used as a reference point in studying populations of women who have been occupationally exposed to known workplace contaminants.

Also from DBRA, scientists collaborating with university scientists in the U.S. and health researchers in Taiwan, published a study in the journal Science on Taiwan women who ingested PCB-contaminated cooking oil in 1978-79. The study followed up on the health effects shown by their children, none of whom consumed the cooking oil, but who were exposed to the residual body burden of PCBs and the PCB contaminant dibenzofuran, transplacentally. These children showed an array of physical health effects as well as behavioral and developmental delays, as compared to a control group.

From the Division of Intramural Research, the Laboratory of Molecular Carcinogenesis, investigators have published in the journal Science a study of the carcinogenic mechanism of arsenic, long puzzling because of its established role in human cancers, but its failure to cause tumors in laboratory animals. Evidence from this study strongly suggests arsenic may have activity important after initiator and promotor agents have already acted on cells. Thus, arsenic may act as a "tumor progressor," causing amplification of a certain oncogene, and leading to cancer. This study has relevance to arsenic toxicity and mechanisms of carcinogencity in general.

A study funded by the Division of Extramural Research and Training re-emphasized the current concerns about lead toxicity, indicating that elevated lead levels in children are present across a broad demographic cross section and not just in poverty stricken pockets. Also, the study showed that health effects were apparent at blood lead levels below those considered permissible under present regulations.

The Division of Toxicology Research and Testing continues to broaden its toxicological characterization of chemicals nominated and selected for short-term studies, and two-year animal studies. Enhanced experiment design, a highly developed quality assurance program, exceptional pathology work group teams, and a rigorous system of peer reviews of technical reports insure that the NIEHS support of the National Toxicology Program makes NTP a world resource for toxicology research.

DTRT and DBRA investigators published a study in the journal Science comparing results of selected short term carcinogenicity studies with the results of two-year rodent studies, to determine if a specific short term test, or battery of short term tests could successfully predict for results in the two-year rodent studies. The investigators concluded that there was substantial agreement between the short term tests, but that no one short term test, or battery of such tests, could predict with high enough accuracy to eliminate the need for the two-year rodent studies. The study is an example of continual collaboration between Divisions at NIEHS, and the central role of NIEHS in the fields of toxicology and environmental health sciences.

The NIEHS' central place in toxicology research is also illustrated by the five international meetings held during March and April 1988. These covered diverse subjects and brought together scientists from throughout the United States and the world. The meetings were on the calcium messenger system and its implications for toxicology research; benzene metabolism, toxicity and carcinogenicity; environmental health in the 21st century (followed by a hearing of the Congressional Subcommittee on Natural Resources, Agriculture Research and Environment); the toxicology, carcinogenesis, and human health aspects of 1,3-butadiene; and the conference on research advances in prenatal craniofacial development.

Other areas of rapid advances in NIEHS research, both by staff scientists and through grants, include studies of oncogenes, their mechanisms and relation to environmental contaminants; the ongoing Harvard study relating air quality in selected cities to human health; the use of nuclear magnetic resonance spectroscopy and imaging, to study toxicity; and the study of estrogenically active contaminants and their effects on health.

This report gives a detailed account of the broad spectrum of research pursued by NIEHS as it approaches the beginning of the 21st century. A relatively young institute, having celebrated its 20th anniversary in 1986, NIEHS has developed the staff and resources to sustain cutting-edge scientific research on the health effects of environmental agents as these health concerns continue to demand public attention and policy decisions.

INTERNATIONAL PROGRAMS

INTERNATIONAL PROGRAMS
OFFICE OF THE ASSISTANT TO THE DIRECTOR FOR INTERNATIONAL PROGRAMS
Summary Statement

The Office of the Assistant to the Director for International Programs is responsible for the following areas:

I. INTERNATIONAL PROGRAMS

NIEHS conducts scientific exchanges and collaborative research through a variety of mechanisms that extend from informal contact between investigators in a number of countries to formal bilateral and multilateral agreements for cooperation between the U.S. and the governments of other countries.

Cooperation with the World Health Organization (WHO)

NIEHS has been designated by WHO as a Collaborating Center for Environmental Health Effects since 1975. As a Collaborating Center, NIEHS provides advice and scientific expertise to WHO headquarters and WHO Regional Offices, and assists them in formulating research programs related to the biomedical aspects of environmental pollution.

In 1979, WHO established the International Programme on Chemical Safety (IPCS) a cooperative undertaking involving WHO, the United Nations Environmental Programme, the International Labor Organization, and their Member States. In October 1980, a cooperative agreement was signed between NIEHS and WHO, and NIEHS assumed the function of a Participating Institution (PI) within the IPCS for such activities as international evaluation of the biological effects of chemicals and health hazard assessments, and review and/or validation of methods for toxicity testing risk assessment. In September 1986, the Agreement was extended for another three years. A WHO Interregional Research Unit (IRRU), housed at NIEHS was established in 1981 to assist the Central Unit established at WHO headquarters in coordinating the activities of the IPCS/PI's.

The objectives of the IPCS are: (1) to encourage international cooperation in the evaluation of the effects of chemicals on human health and on the quality of the environment; (2) to coordinate chemical testing and toxicological research to eliminate unnecessary duplication of efforts; (3) to develop international protocols for laboratory testing, epidemiological studies, and risk assessment; (4) to develop international guidelines and exposure limits for chemicals in air, water, and food and limits for hazardous chemicals in workplaces; (5) to develop response mechanisms for coping with chemical emergencies which may be international in scope; and (6) to promote training and development of manpower in areas of specialties necessary for the achievement of program goals. The IPCS provides an effective multilateral mechanism for dealing with global environmental health issues and avoiding costly duplication of national efforts to test and assess chemicals.

Since the inception of the Programme, numerous scientific experts from NIEHS have participated on IPCS committees, special consultations, conferences, and technical working groups. The Director, NIEHS, serves as a member of the IPCS Programme Advisory Committee (PAC) and chaired the PAC during the Programme's first two years of operation. This Committee, composed of members designated by the Director-General of WHO, is the general oversight body providing advice on the policies and priorities of the IPCS. During 1988, the Assistant to the Director, NIEHS, participated in a special IPCS consultation on the "Priority Setting of Chemicals."

Also, during 1988, NIEHS hosted a Meeting of Directors of IPCS Participating Institutions. Over 60 people from 23 countries participated in this meeting to review the progress and determine future directions of the IPCS.

A number of NIEHS scientists continue to play a leading role in the IPCS Collaborative Study on Short-Term Tests for Genotoxicity and Carcinogenicity. Institute scientists also participated in the preparation of the monographs on (1) Principles and Methods for Evaluating the Neurotoxicity of Chemicals; (2) Toxicokinetics; (3) Principles and Methods for Evaluating the Toxicity of Chemicals - Part II; (4) Manual on Epidemiological Methods; (5) Approaches Needed to Establish the Role of Chemical Agents in the Etiopathogenesis of Certain Non-Communicable Diseases and (6) Immunotoxicology. Also, during 1988, NIEHS staff continued to review IPCS criteria documents, working papers, and proposed projects.

During FY 1988, NIEHS staff also participated in the International Meeting of the Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC) dealing with "Methods to Assess the Adverse Effects of Pesticides on Non-Vector Targets," to be held in Czechoslovakia. SGOMSEC is an IPCS activity sponsored jointly with the Scientific Committee on Problems of the Environment of the International Council of Scientific Unions.

NIEHS also collaborates with the WHO International Agency for Research on Cancer (IARC). Collaborative efforts include the establishment of a registry of workers exposed to particular pesticides which contain dioxin contaminants. During FY 1988, scientists from NIEHS and the U.S. National Toxicology Program (NTP) participated in a number of IARC sponsored expert working groups to prepare "IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans." These monographs collect all available relevant experimental and epidemiological data about a chemical or groups of chemicals to which humans are known to be exposed, and summarize the evidence for the carcinogenicity of chemicals and other relevant information.

US-China (Mainland) Cooperation

Cooperation between the United States and the People's Republic of China in the area of environmental health was initiated during 1980 under the US-PRC Agreement for Cooperation in the Science and Technology of Medicine and Public Health. NIEHS is a participant in the topic on public health and health services research, which includes concerns relating to environmental and occupational health. Discussions between both sides have been held during exchange visits centering around cooperation in the following areas: reproductive and developmental toxicology, validation of short-term test methods to detect and assess carcinogens, mutagens, and teratogens in the environment; and the extrapolation of laboratory animal data to man. NIEHS scientists have given research seminars at various institutions in the People's Republic of China, and have hosted the visits of numerous Chinese scientists to discuss research programs of mutual interest. During 1988, the Director, Division of Biometry and Risk Assessment, NIEHS, visited P.R.C. to initiate a collaborative study investigating the activation of oncogenes in response to various etiologic exposures for human lung and liver cancer.

US-China (Taiwan) Cooperation

Collaborative studies between U.S. and Taiwanese scientists are carried out through a Cooperative Program in the Biomedical Sciences between the American Institute in Taiwan and the Coordinating Council for North American Affairs. For the past six years, NIEHS scientists from the Biometry and Risk Assessment Program have collaborated with Taiwanese scientists in studies investigating the effects of accidental human exposure to polychlorinated biphenyls (PCBs) in Taiwan. Studies on the effects of PCB exposure on enzymatic activity in human tissues suggest a potential for substantial and persistent effects of these pollutants on human metabolism. NIEHS epidemiologists in collaboration with Taiwanese scientists are conducting a clinical evaluation of a cohort of children exposed transplacentally to PCBs and related chemicals. Preliminary data show that the children have abnormalities of pigment, nails, teeth, and skin; and show a developmental delay on a number of dimensions. The data is being analyzed and further followup of this cohort is being considered.

US-Egypt Cooperation

Cooperation between American and Egyptian environmental health scientists is being carried out under the auspices of a U.S.-Egypt Joint Working Group on Health Cooperation (JWGHC), supported by the U.S. Agency for International Development. NIEHS has been assigned responsibility for the U.S. Subcommittee on Environmental and Occupational Health of the JWGHC. Efforts are continuing in the establishment of an Information Unit for Environmental Impact Assessment. This Information Unit, located at the Environmental Health Center in Cairo, provides the Egyptian Ministry of Health and Egyptian institutions and universities with access to U.S. information on environmental and occupational health hazards, thus strengthening the scientific base for research and regulatory decision making.

US-Finland Cooperation

A Memorandum of Understanding on Collaboration between NIEHS and the Finnish Institute of Occupational Health, Helsinki, was formalized in November 1982. Since then a number of exchange visits have taken place between U.S. and Finnish scientists to exchange information on pharmacokinetics, reproductive toxicology, neurobehavioral toxicology, genetic toxicology, epidemiology and risk assessment, and strategies for toxicological research priority settings.

US-India Cooperation

NIEHS and NTP scientists have been cooperating with India by conducting experimental animal studies on the toxicity of methyl isocyanate, the chemical responsible for the world's worst industrial disaster which occurred in Bhopal, India in December 1984. During 1988, several NIEHS scientists visited research and government organizations in India to discuss recent NIEHS research activities. NIEHS also hosted the visits of a number of scientists from India to discuss toxicological research activities.

US-Italy Cooperation

Cooperation in environmental health research between American and Italian health scientists has taken place since 1977 under a Memorandum of Understanding between the U.S. Department of Health and Human Services and the Italian Ministry of Health. Since then a number of exchange visits have taken place to exchange information on the bioavailability of 2,3,7,8-tetrachlorodibenzodioxin, studies on the chemical contamination of drinking water, chemical selection procedures, and the design of two-year toxicity studies. During 1988, a NIEHS scientists participated in a small workgroup in Italy to determine the feasibility of studying the children exposed to dioxin during the Seveso incident in 1976.

US-Japan Cooperation

Cooperation between American and Japanese scientists on environmental health problems takes place under two formal agreements: The US-Japan Cooperative Medical Sciences Program and the Agreement on US-Japan Cooperation in Research and Development in Science and Technology. Under the US-Japan Cooperative Medical Sciences Program, American environmental health scientists participate in the Panel on Environmental Mutagenesis and Carcinogenesis. The Director, Division of Biometry and Risk Assessment, NIEHS, serves as a member of the Panel; and the Assistant to the Director for International Programs, NIEHS, serves as the Panel's program officer. Joint areas of research focus on the detection of mutagenic and carcinogenic chemicals using both *in vitro* and *in vivo* test systems, and on monitoring human populations for evidence of exposure to mutagenic and carcinogenic chemicals. The Director, NIEHS, serves as a State Department appointed member of the Joint Committee which oversees the overall activities of the US-Japan Cooperative Medical Sciences Program. This Joint Committee meets annually, alternating between the U.S. and Japan. During FY 1988, the Director, NIEHS; the Director, Division of Biometry and Risk Assessment; the Head, Molecular Toxicology Section, NIEHS; and the Assistant to the Director for International Programs, NIEHS, participated in the 16th US-Japan Joint Environmental Panel Conference on "Molecular Approaches to Environmental Mutagenesis and

Carcinogenesis" held in Yokohama, Japan. Ten U.S. participants and sixteen Japanese participants gave presentations on oncogenes, tumor promotion, and tumor suppression. Also, during 1988, the Director, NIEHS, participated in the US-Japan Joint Subcommittee Meeting on Program Review and Planning, and the 24th US-Japan Joint Committee Meeting.

Under the US-Japan Agreement on Cooperation in Research and Development in Science and Technology, NIEHS participates in the toxicology program area in the counterpart working group on health. Cooperative activities under this agreement focus on the testing of chemicals for mutagenic, carcinogenic, and other toxic effects. In FY 1988, annual exchange visits between Japanese and NIEHS scientists continued, and both sides have shared information on test method development, which chemicals will be tested, and test results. Several test systems are being reviewed and validated in both U.S. and Japanese laboratories, and collaborative activities on oncogenes have been initiated. Joint annual scientific symposia are also planned.

US-USSR Cooperation

Collaboration between Soviet and American environmental health scientists is carried out under the auspices of two cooperative agreements between the United States and the Soviet Union. Under the Medical Science and Public Health Cooperative Agreement, scientists from both countries are conducting joint research on the biological effects of low frequency electromagnetic radiation. 1988 was the sixteenth year of formal collaboration in environmental health research between the U.S. and U.S.S.R. Cooperative research efforts have involved formal workshops and exchange visits between scientists of both countries. Duplicate collaborative experiments aimed at evaluating sensitive tests for determining the biological effects of electromagnetic fields on the nervous system and to validate research results obtained in the U.S. and the Soviet Union have been completed. A large amount of scientific information has been exchanged during these collaborative activities.

NIEHS also participates in the US-USSR Agreement on Cooperation in the Field of Environmental Protection which is administered for the United States by the Environmental Protection Agency. The Director, NIEHS, serves as DHHS representative to the Environmental Protection Agreement and co-chairman of the working group concerned with the biological and genetic effect of pollution. Exchange visits under this Agreement have been conducted in research areas concerned with the mutagenic effects of environmental contaminants.

US-Yugoslavia Cooperation

Under the auspices of the US-Yugoslavia Joint Board for Scientific and Technological Cooperation, NIEHS scientists continued collaborative studies in 1987 on the evaluation of the genetic effects of low levels of environmental chemical mutagens in bacterial systems, and comparison with eukaryotic cells.

II. INTERAGENCY COORDINATION

A number of federal and state agencies are involved in collaborative efforts to establish integrated systems for gathering, evaluating, and disseminating information on the health and environmental effects of chemical substances. The Assistant to the Director for International programs represents NIEHS on the Subcommittee on Information Coordination of the DHHS Committee to Coordinate Environmental Health and Related Programs (CCEHRP). This committee identifies the needs and establishes the mechanisms for the collection, storage, and dissemination of toxicologic information within DHHS.

The Comprehensive Environmental Response, Compensation and Liability Act of 1980 (CERCLA), provides for several Federal organizations to participate in a coordinated response to provide information and advice on health hazards resulting from chemicals released into the environment and from the cleanup of hazardous waste disposal sites. In order to provide for the effective coordination of the collection, development and evaluation of the information necessary to determine the potential health hazards associated with such chemicals, the DHHS Committee to Coordinate Environmental Health and Related Programs established a Hazardous Waste Information Evaluation Subcommittee (HWIES). The Assistant to the Director for International Programs represents NIEHS on this Subcommittee. The HWIES, composed of technical experts from various DHHS agencies, evaluates the available information on a number of chemicals frequently found in waste dumps and makes recommendations concerning the testing of these chemicals by the National Toxicology Program, and structured data record creation by the National Library of Medicine. The Assistant to the Director for International Programs also coordinates the NTP's review of and identification of research needs identified in the toxicological profiles prepared under the Superfund Amendments and Reauthorization Act of 1986, and is a member of the Technical Advisory Committee of the Governor's Waste Management Board of the State of North Carolina.

FACILITIES ENGINEERING BRANCH

FACILITIES ENGINEERING BRANCH
Summary Statement

Responsibilities:

The Facilities Engineering Branch (FEB) plans, directs, supervises, and coordinates all facilities engineering activities including, but not limited to, engineering design, inspection, construction, master planning, operation of utility plants and related systems, maintenance and repair of all real property (buildings, grounds, surfaced areas, utility plants and systems), maintenance of facility operations equipment, fire prevention and protection, custodial, refuse collection and disposal, and storage and supply of construction and operations materials.

As directed, FEB performs other mission support activities including, but not limited to, security and the design, fabrication, alteration and repair of scientific instrumentation.

FEB has four sections under the Office of the Chief.

(1) The Facilities Management Section (FMS) is the coordination point for all service requests and work orders, providing planning, estimating, scheduling, expediting, and contracts management.

(2) The Engineering Design Section (EDS) provides architectural and engineering support required for planning new facilities and improvements, major alterations and repairs to existing facilities through either in-house design or contract with Architectural/Engineering firms. EDS also administers and inspects construction performed under contract.

(3) The Special Projects Section (SPS) coordinates planning and design for construction of an addition to Building 101, related site improvements and utilities systems expansion on the South Campus, as well as other special design and construction projects.

(4) The Facilities Maintenance and Operations Section provides construction, renovation, repair service and maintenance and storage of shops materials and parts for all NIEHS buildings, utilities systems and installed equipment at both North and South Campuses. Trades include carpentry, locksmith, masonry, plumbing, pipefitting, sheet metal, welding, painting, utility system repair/operator, and electrical. The Maintenance and Operations Section also provides 24 hour a day service to South Campus buildings, monitoring all environmental systems through the JC-80 computer system.

Accomplishments:

North Campus Improvements: Our largest single project was the $225,000 renovation of Building 14. Laboratories were repaired and altered, a tissue culture lab was established, rooms were prepared for a two-year inhalation study of methylene chloride, and floor surfaces were sealed. Minor repair and alteration projects were accomplished in other facilities. Anticipated is an FY-88 award of a contract to reroof and restain Building 18.

South Campus Improvements: Building 101 "A" basement was provided with underfloor power and telephone wiring and occupied by offices of Safety, the Division of Intramural Research, and the Division of Toxicological Research and Testing. The Computer Room, A-363, received a major upgrade. Air conditioning capacity was doubled and a fly wheel generator installed to insure uninterruptible power. Additionally, a fireproof room was constructed in the Supply Warehouse (Building 104) and the Chemical Storage Building (103) for the Safety Office to segregate and handle hazardous chemicals and wastes.

Completion of design for the Additions to Building 101 and Support Facilities has been delayed because of insufficient funds. Present funds are adequate to continue design through the Intermediates (70 percent) phase of design. Funds to complete design and construction are included in the NIH Fiscal Year 1990 Building and Facilities budget submission. The project will add some 57,500 square feet of laboratory facilities and 29,700 square feet of office space. Completion of the project will allow NIEHS to consolidate its activities on the South Campus.

In the service contract area, our greatest accomplishment is the award and putting into service the Jones Operations and Maintenance Contract for service to the North and South Campuses power plants and utilities. Having started on November 1, 1987, we have overcome several problems not usual in a new service type contract. The Preventive Maintenance to be accomplished under the contract is a much needed function for the facility and is expected to lessen the breakdown maintenance requests from the research community.

Likewise, some of our other service contracts or arrangements were reestablished during the year to include the Preventive Maintenance requirements. Goodwin Refrigeration is now doing refrigeration preventive maintenance and repair under a fixed price arrangement. That is, the labor and the materials for all repairs and PM are at a fixed monthly cost to the Government. Our elevator maintenance and repair is done in the same manner. As with the Jones O&M contract, the Preventive Maintenance requirements for the equipment is established by the Government, but the check sheets and instructions for the PM are routinely provided and recorded by our equipment inventory contractor.

Our Equipment Inventory Contractor, Computer Related Services, continually inventories our equipment at the Institute and adjusts and provides the preventive maintenance requirements for the specific contractor responsible for the area of service, as well as PM accomplished by Government staff. In addition, they schedule the equipment PM over the year to level out the manpower effort. The overall Preventive Maintenance Program is operating very well. One of our service contractors, who admittedly had doubts when his contract was awarded as to the fixed pricing arrangement, has reported to us that he estimates that the Preventive Maintenance Program for his contract has cut his corrective maintenance requirements by as much as 40 percent.

Our organization has become more efficient with experience. The Planner/Estimators (PE's) have established a rapport with the research community in being better able to help them accomplish renovations and improvements in their work areas. The PE's have resources to get the job done in many ways. Some work is accomplished by use of our own shops trades, and some tasks are done by outside contractors through a purchase order arrangement or through the Task Order Contractor. Best of all, the PE's can take a task/job and follow it from beginning to end, both overseeing the desires of the requester and being the link of communications for the various crafts involved in the task. The research and laboratory personnel have repeatedly expressed their appreciation for having one of our staff help lay out a floor plan for the casework and work area and then working out the details to have the work accomplished.

The space management portion of our activities has been called on repeatedly during the past months. Drawings of the space assignments by floor for each building have been prepared and provided to the appropriate person. These color-coded plans have been greatly appreciated by the users in that they are easily interpreted as to who is in what space.

Following are some of the tasks/jobs that have been accomplished:

Reconfiguration of six exposure chambers in Building 14.

Renovation of the Cafeteria in Building 10, including new carpet, complete painting, plants, and niceties to make the area more appealing.

Relocation of two walk-in boxes from a laboratory in Building 13 to the breezeway at the dock of Building 16. This included not only the relocation of the boxes, but also upgrading and refurbishing the equipment.

Renovation or modification of several laboratories in Buildings 1, 3, 5, 6, 7, 13, 14, and 101.

Removed trees on the North Campus which had been infested with Pine Bark Beetles and burned them in our incinerators at Building 106.

Erected a fence around the radiation area of Building 4A to keep people beyond the 5 gauss line of the magnets.

Planted trees at the entrance to South Campus.

Planted flower beds for the Olympic Festival activities in the area.

Rebuilt the Softball Field.

Installed gas meters on the Pathological Incinerators in Building 106.

Installed carpet in the elevators in Building 101 and brought elevators up to the North Carolina State Code requirements.

Waterproofed Module E north wall of Building 101.

Repaired the reverse osmosis water system in B Basement of Building 101 and placed it back in service.

Completely repaired the lawn sprinkler system on South Campus and turned it over to the contractor for operation.

Placed all animal room lights in Modules C, D, and E, Building 101, on the emergency power system.

Refurbished the animal colony and made it ready for accreditation inspection. Changed out and repaired the water deaerators in the basement of Module B, Building 101.

Updated the security locking system in Building 15.

Repiped the animal cage, rack washing equipment and the glassware washers, Building 101.

Repiped the air compressors in Module D, Building 101, so that they can be used as backup for the Ducon air control system.

Upgraded and painted the chilled and hot water pumps and Kathabar tanks in the basement of Module B, Building 101.

Installed adjustment jacks on all motor mounts and installed lights in fan housings of all air-handling units in C, D, and E Penthouses, Building 101.

Refurbished basement of Module E, Building 101, to prepare it for laboratory animal habitation.

The following addresses the accomplishments and future goals for FEB computer applications and usage.

FEB utilizes a departmental VAX computer for support of its most active computer applications and systems. This VAX computer was updated with new releases of system software and other manufacturer software products as dictated by system utilization at FEB. Equipment was installed at the FEB to provide for a high-speed communication link between most FEB computer users and the VAX cluster in Building 101. This equipment has the capacity to support up to 16 FEB computer users accessing the VAX cluster in Building 102 at higher communication speeds.

Development of computer generated floor plan displays and work space layouts representing information maintained within the system was developed utilizing special graphics software and IBM/PS2 computers. A specialized scanning device was used to convert floor layout drawings into formats for computerized processing. This graphics package is also used for work request planning of FEB work requests.

The FEB computer system for tracking contracts and producing Record of Call and 2345 payment documents was modified to meet extensive new Contract Management Office requirements for processing Record of Calls when used for payment on a Blanket Purchase Order contract. The Contract Management Office is considering implementing this FEB system throughout the Institute. The FEB began utilizing IBM/PS2 computers for word processing by most FEB sections. The IBM/PS2 computers replaced the DEC Rainbow units that had been previously used.

Stockroom accountability for bench stock material was implemented in the Stockroom and Work Order Systems. This system feature added another dimension in tracking material usage at FEB.

Is is planned for the FEB to institute Bar Code processing of the stockroom materials. FEB has for some time desired this capability and this next year, bar coded materials and the necessary computer support for bar coding may become a reality. FEB has passed on their bar coding expertise to several other potential areas at NIEHS.

Plans:

With the substantial curtailment of funds experienced in FY-88, projects to repair the Building 101 air conditioning, to repair water pumps and install isolation valves, to convert our high temperature hot water system to a variable flow method, and to construct a laboratory floor in E Module were all unfortunately delayed until at least the FY-89 budget year. We look forward to much greater design and construction activity in the coming fiscal year.

We are either currently working on or plan to participate in accomplishing the following:

Better organize the method in which we pass out parking permits for visitors to the Institute. There have been problems with vendors and service companies which routinely come to the Institute, and the Security contractor has requested assistance in control of parking.

A new drawing package is to be implemented to improve the Planner/Estimators capacity and also to provide the Space Management Program with better drawings.

The Incinerators in Building 106 may need modification to meet the State or EPA certification requirements.

Upgrade the Delta 1000 Monitoring System on the North Campus.

Repaint traffic stripping of NIEHS streets and parking lots.

Install electronic card reader type security system in Building 10.

Repair floors in C, D, and E Basements of Building 101.

Renovate Building 101 Cafeteria exhaust washdown system.

Repair South Campus building facings expansion joints.

HEALTH AND SAFETY BRANCH

HEALTH AND SAFETY BRANCH

Summary Statement

The NIEHS Health and Safety Branch is administratively located within the Office of Occupational Health and Technical Services, Office of the Director and has broad responsibility for chemical and radiation safety, physical safety, fire protection, emergency preparedness, environmental protection and occupational health surveillance. The Health and Safety Branch conducts research studies and special investigations relative to specific safety concerns.

Toxicological research laboratories pose unique health and safety problems due to the great variety of chemicals and radioisotopes that may be used and the non-routine nature of many laboratory procedures. The primary emphasis of the NIEHS Health and Safety Branch is to minimize exposures and unsafe conditions through utilization of containment equipment, following appropriate work practices and procedures and use of personal protective equipment. The primary tools for accomplishing this objective are the Institute's hazard communication program, required hazardous agent safety protocols, employee training and programs for information dissemination.

Chemical Safety/Industrial Hygiene

Programs for safe use of hazardous chemical agents are high priority at NIEHS. There were approximately 166 active protocols for use of hazardous chemicals in force during FY88. An important component of the Health and Safety Program is routine surveys of all laboratories, shops and warehouses on a periodic basis. These surveys serve to identify potential hazards and to initiate preventive actions as well as to maintain an awareness of potential hazards. Use of primary containment devices and other engineering control measures is the preferred means of minimizing occupational exposures. All laboratory hoods, a total of 135, are inspected and their performance measured on a quarterly basis. Information concerning deficient hoods is immediately referred to the Office of Facilities Engineering for corrective action. In addition to laboratory hoods, a total of 65 biological safety cabinets are in use at NIEHS. All biological safety cabinets are tested and certified annually by an independent testing firm.

During FY88 the Health and Safety Branch continued to expand and improve programs for monitoring occupational exposures. Workplace air samples for evaluating a variety of potential exposures such as organic vapors, wood dust, formaldehyde and nuisance dust were collected as part of routine sampling programs and in response to specific requests. Exposure monitoring for formaldehyde and benzene increased during FY88, because of the new standards issued by Federal OSHA. Special air sampling studies were completed to evaluate hard wood dust exposures among animal bedding handlers. During FY87 an industrial hygiene study of exposures experienced by animal handlers was completed in conjunction with an ongoing surveillance program for laboratory animal allergies. These samples were analyzed for specific animal antigens through an interagency agreement which was established with the National Institute for Occupational Safety and Health (NIOSH). This study continued during FY88 with collection of additional high volume area samples for quantitative antigen determinations. A manuscript concerning the animal allergy study was jointly published with NIOSH in FY88.

The Industrial Hygiene Laboratory continues to be an integral component of Health and Safety Branch programs. As a part of the industrial hygiene laboratory quality control program, NIEHS participates in the NIOSH Proficiency Analytical Program (PAT). During FY88 PAT samples for various organic solvents and asbestos were analyzed on a quarterly basis to evaluate laboratory quality control procedures. The NIEHS laboratory continues its high proficient rating for each round.

The primary emphasis of NIEHS health and safety programs is minimizing personnel exposure through proper experimental design and use of laboratory containment equipment. Personnel protective equipment is used to supplement other preventive measures. A written respiratory protection program has been developed which includes initial selection criteria, qualitative fit testing, training of new users and annual maintenance checks by the Health and Safety Branch. There were approximately 66 occasional users of respirators at NIEHS during FY88. In addition to qualitative fit testing, respirator users are given a complete overview of the respiratory protection program. An annual inspection and maintenance of respiratory protection equipment is conducted by the Health and Safety Branch to insure that broken, worn, or deteriorated parts are replaced and filters and/or cartridges are changed as indicated.

The Occupational Safety and Health Administration (OSHA) adopted the Hazard Communication Standard on November 25, 1983 for the purpose of ensuring that chemicals are evaluated for their hazards by chemical manufacturers and importers and that these hazards are communicated to employers and employees in the manufacturing sector. The State of North Carolina has adopted the federal standards and in addition has added significant amendments. These amendments are included in the N.C. Occupational Safety and Health Hazard Communication Standard and the N.C. Hazardous Chemical Right to Know Act and went into effect on June 27, 1985. In compliance with these regulations, NIEHS conducted a detailed chemical inventory and supplied the local fire marshal with a list of chemicals stored on-site in quantities greater than 55 gallons or 500 lbs. A detailed analysis of these regulations as well as options available for NIEHS compliance was completed during FY86. Training for the hazard communication program continues and it has been integrated into the Laboratory Safety Course. Collection of material safety data sheets, as part of the hazard communication program, continues to be done by a contractor. The system will utilize computer imaging technology for storage of material safety data sheets. The computer based system is expected to be operational within the Health and Safety Branch in FY89.

Radiation Protection

Use of radioisotopes and radiation sources is an integral part of biomedical research. The Institutes' use of radioisotopes remains high with about 250 active protocols for the use of radioactive material. Approximately 1900 shipments of radioactive material were received in FY88.

Routine duties of the radiation protection program include monthly laboratory surveys, surveys of sealed sources, checking for contamination in cases of suspected spills, receiving and distributing incoming isotopes, calibration of radiation detection instruments, disposal of radioactive inventory of all radioisotopes at the institute. In addition to these routine duties, special investigations are conducted to address specific issues relative to NIEHS radiation safety programs.

Incineration has been a vital method for radioactive waste reduction and treatment for several years and is becoming even more important as other alternatives are being reduced or eliminated. In 1988 we completed a study on the incineration of 35S. This work compliments previous work done on the incineration of 14C and 3H wastes. A study was conducted on the light sensitivity of a finger-tip dosimeter for beta radiation that was developed here. Ongoing evaluations are being made on the use of biodegradable liquid scintillation fluids and plastic vials in order to reduce the volume of hazardous waste or make it more amenable to incineration.

Safety and Health Training

Safety and health training is an important component of the Institute's safety program. The Health and Safety Branch offers a number of courses for laboratory personnel including "Laboratory Safety", "Introduction to Radiation Safety" and "Hazard Communication". Approximately 200 employees attend each of these courses per year. In addition to the above courses, routinely scheduled courses in cardio-pulmonary resuscitation, First Aid and "Fire Extinguisher Use" are made available to all interested NIEHS employees.

Safety and Health programs and training for NIEHS employees other than laboratory workers, such as shops and maintenance personnel continues to be an important area of emphasis. Major training programs include hazard communication, confined to space entry, and lockout/tagout procedures. The confined space program requires the HSB to monitor all confined areas such as valve pits for hazardous conditions on a routine basis and before entry by maintenance personnel. Safety training meetings for Facilities Engineering Branch personnel continued in FY88 with an emphasis on the supervisors providing more input in the training.

During FY88 Jones Operations and Maintenance Company (JOMC) assumed responsibility for building maintenance on North Campus, the power plant in Building 105, and the waste incineration operations in Building 106. The Health and Safety Branch assisted JOMC in developing their safety plan and then assumed the task of monitoring the JOMC safety program. The Health and Safety Branch also provided input into appropriate safety topics for JOMC and assisted in locating training materials.

Fire Protection/Emergency Preparedness/Design Review

Fire prevention and emergency preparedness are components of the overall NIEHS program. The Durham County Fire Marshal continued annual fire inspections for all NIEHS properties. Suggestions made by the Fire Marshal were implemented. An Occupant Emergency Plan was developed for the new East Campus site.

The Health and Safety Branch has become increasingly involved in the review process for building design. Numerous buildings underwent modifications requiring review and approval. Considerable time was spent reviewing drawings and specifications for the new modules which are to be built onto Building 101. The review process involves many consultations with the Facilities Engineering Branch design section.

Occupational Medicine Programs

The Health and Safety Branch has responsibility for providing occupational health services for NIEHS employees. Services are currently provided through an interagency agreement with the PHS, Division of Federal Employee Occupational Health. Services provided include emergency treatment, periodic occupational health surveillance programs, preventive health programs, health promotion and education programs. A special project to develop a surveillance program for laboratory animal allergies was initiated during FY85 and continued during FY86 and FY87. The initial implementation included a questionnaire to determine allergy prevalence among NIEHS employees and initiation of an industrial hygiene study to identify potential exposures to airborne allergens during animal handling. During FY87 a second allergy questionnaire was administered to evaluate allergy incidence. A clinical surveillance program for NIEHS animal handlers was initiated in FY88 and includes initial and follow-up allergy questionnaires, RAST testing.

The Employee Assistance Program which was developed and implemented in FY86 continued during FY87 and FY88. This program provides employees with assistance in dealing with psychological or emotional problems potentially affecting work performance. During FY88 use of the Employee Assistance Program continued to grow with approximately 50 new users.

Workmen's Compensation

The Health and Safety Branch is responsible for maintaining NIEHS illness and injury statistics and for managing claims submitted to the Federal Office of Workmen's Compensation Programs. During FY88 NIEHS injury and illness data for FY87 were analyzed. NIEHS injury and illness incidence rates were found to be 3.5 OSHA reportable cases per 100 employees and 1.3 lost work day cases per 100 employees. These rates are significantly lower than comparable rates for the Federal sector and slightly lower than NIEHS rates for FY86. Results of these analyses will be used to direct future prevention programs at the Institute.

Hazardous Waste Management

The Health and Safety Branch continues to seek ways of improving the Institutes' Hazardous Waste Management Programs. An expanded hazardous waste disposal contract was awarded in FY87.

Federal regulations require activities that operate a treatment, storage , or disposal (TSD) facility to obtain a Part B Hazardous Waste Permit. In FY87 NIEHS notified the State of North Carolina concerning its on-site waste treatment and storage activities and is currently operating under interim status as an incinerator of hazardous wastes and as a storer for certain wastes. NIEHS submitted Part B of the permit application in first quarter of FY88. The Part B application is an extensive document consisting of maps, diagrams, plans and facility design information. In addition, to demonstrate appropriate incinerator performance, NIEHS must conduct a trial burn and submit results to the State of North Carolina for review. A contract to develop a trail burn plan and to conduct the trail burn was awarded in the first quarter of FY88.

Environmental Protection

Responsibility for the Institutes' environmental protection programs reside with the Health and Safety Branch. Responsibilities include federal, state and county contact, maintaining environmental permits required by regulatory agencies for compliance, and advising other components at the NIEHS on changes in requirements for regulatory compliance. During FY86 protocols for detailed environmental audits were developed and reviewed. These protocols provide a suitable framework for evaluating and documenting compliance with the wide range of environmental regulations with which the NIEHS must comply. During FY88 the following audits are expected to be completed: hazardous waste management, solid waste management, air emissions, hazardous materials management, and bioenvironmental hazard/toxic materials, handling and disposal. In general, the NIEHS was found to be in compliance with applicable regulations. Problem areas which were identified have been addressed and audit protocols updated where necessary.

In an effort to control and prevent releases from underground storage tanks, Congress passed amendments to the Resources Conservation and Recovery Act (RCRA) called the Hazardous and Solid Waste Amendments of 1984. As part of this new RCRA provision, Congress included requirements for owners of underground storage tanks to notify designated state or local agencies. The notification requirements apply to underground storage tanks that contain regulated substances defined as hazardous under Superfund legislation and underground storage tanks that contain petroleum products. The notification forms for the NIEHS storage tanks were completed in February of FY86 and sent to the designated state agency.

In addition to the above requirements for underground storage tanks, pending EPA legislation will require that all existing underground storage tanks be tested for integrity. While not currently required by regulations, all the NIEHS underground storage tanks were tested for leaks. Several tanks on the North and South Campuses have not passed the initial leak test and were further investigated. These investigations suggested a long standing problem with the six 20,000 gallon power house tanks requiring remedial action. Remedial action plans were developed and implemented. An above ground oil storage facility was proposed and design initiated in FY88.

LIBRARY AND INFORMATION SERVICES BRANCH

LIBRARY AND INFORMATION SERVICES BRANCH
Summary Statement

The NIEHS Library is the principal science reference resource for the Institute. Library and information services include reference services, computerized literature searching of bibliographic and scientific databases, maintenance of a collection of 700 periodical titles and 20,000 books on environmental health, participation in a nation-wide network for interlibrary loan and cataloging, procurement of 2,000 new books for the Library and the laboratories, and publication of a monthly newsletter and the annual bibliography of publications by NIEHS personnel. Microcomputers and large automated systems play an integral role in every functional area in the NIEHS Library.

Mission: The Library (1) Plans and provides specialized library and information services to NIEHS and NTP scientists and administrators; (2) provides reference service and subject searches using computerized bibliographic databases; (3) acquires, organizes and maintains a broad collection of library materials; (4) provides professional assistance to NIEHS employees and visitors in optimum utilization of library resources; (5) plans and maintains the NIEHS archives and repository for experimental data; (6) provides procurement services to NIEHS managers and staff for books, journals, and interlibrary loans; (7) provides leadership and consultative services in library-type areas including database management and information storage and retrieval.

Reference/Literature Searching: The Library maintains one of the most advanced computerized literature searching capabilities in the world, with access to more than 600 databases covering subjects from toxicology to public administration. During FY88, Library personnel performed comprehensive multi-database searches on some 3,800 topics for Institute investigators and administrators. This was an increase of 13% over last year. Part of the increase was due to the assignment of full responsibility to the Library for doing comprehensive toxicology searches for the TRTP chemical managers. Besides online searches, 13,000 other reference questions were answered. The most heavily used online databases continued to be TOXLINE, MEDLINE, Hazardous Substances Data Bank, Biological Abstracts, and Chemical Abstracts. Examples of search requests include the following:

- Delphi studies relating to environmental health in the 21st century. (OD)
- The relationship of aluminum to Alzheimer's Disease. (OD)
- The effect of aging on reproductive hormones. (DIR)
- The influence of lymphokine activation of B lymphocytes on phosphatidyl inositol metabolism. (DIR)
- Toxicity testing results of aldicarboxine. (NTP)
- The effects of antibodies in animal feeds on human health. (DTRT)
- Mathematical models of carcinogenesis. (DBRA)

The Library introduced "COMPSEARCH," an easy way for scientists to request literature searches over the Institute's VAX. In addition, Library Search Analysts sent search results back to requestors using electronic mail and the VAX computer instead of paper printouts.

The Library staff began the fourth year of training investigators to do their own online literature searches. The number of trainees increased from 80 to 110 on the PaperChase System, a user-friendly version of MEDLINE. Now, NIEHS scientists can choose between having searches done by Library Search Analysts or doing the routine online searches themselves.

NIEHS Information System: The Library in conjunction with OPPE embarked on a new project in FY88, an Automated Research and Studies Information System (ARSIS). Using an IBM PS2 microcomputer, Library and OPPE staff developed a database of all the research projects being conducted at NIEHS. The Library staff contributed by developing the database structure and doing the subject indexing of the scientific projects. The ARSIS system is used to track the Institute's research activities and expedite responses to Congress and others regarding the Institute's research in specified subject areas.

Journal Collection: The medium of the journal continues to be the primary means of disseminating scientific information. The Library subscribed to approximately 700 periodicals during FY88 and ordered 400 subscriptions for the various laboratories. Issues were checked in and missing ones claimed using a new microcomputer system. The Library continued to bind journals selectively or replace them with microfilm to save space; 713 reels of microfilm were purchased to extend the collection which now includes 16,000 journal volumes and 2,402 microfilm reels.

Book Collection: Continuing the development of the book collection, the Library ordered and cataloged 2,000 books in FY88, of which 27% were ordered for the Library and 73% for the laboratories. The Library also ordered more than 300 government reports.

Computer Catalog: FY88 was the sixth year of operation for the C.L. Systems LIBS 100 computerized catalog and circulation system. This computer supports an online catalog of the books in the Library and in the Labs and is searchable by author, title, or subject using terminals in the Library or any terminal in the labs or offices. This makes it much easier for Institute scientists to find out what books are in the Library. The system also speeds up the check-out procedure, produces overdue notices, and provides statistical reports for management purposes. During FY88 the system was used to check-out 300 books per month. An acquisitions software module was used for book-ordering.

The Library continued using the automated cataloging system, OCLC, a computerized union catalog of books held by more than 7,000 libraries nationwide. The NIEHS Library has experienced a tremendous savings in time owing to the 95% hit rate for new books which already have cataloging data on OCLC. Through an interface, catalog records are transmitted from the OCLC computer in Ohio to the LIBS 100 computer in the NIEHS Library where they are immediately integrated into the public catalog.

Interlibrary Loan: The number of photocopy and loan requests increased by 30% in FY88 to 33,000. This dramatic increase in the number of requests can be accounted for by the assignment of full responsibility to the Library for obtaining copies of articles for DTRT and the NTP. Also, the introduction of a half-time contract employee to do Library photocopying promoted efficiency and improved turnaround time, which resulted in increased usage of the service. The computer program, "INTERLIB," on the Institute's VAX also contributed through its ease of use for sending requests electronically to the Library. For the sixth year in a row, more of the requests were filled from the Library collection (70%) than from other libraries through interlibrary loan (30%). This reflects the ongoing improvement in the NIEHS collection. In addition, the Library made "vendacards" available which could be used by Institute employees who wanted to do their own photocopying at the UNC, Duke and N.C. State libraries. The OCLC computerized catalog also proved useful for verifying titles for interlibrary loan and for locating libraries from which to borrow books throughout the U.S. The NIEHS Library provided 2,400 loans or photocopies to other libraries or to individuals in the Research Triangle Park area. This 85% increase over last year reflects the growing importance of the NIEHS Library as a national resource.

Institute Manuscripts and Bibliography: The Library continued to maintain the NIEHS archives of manuscripts submitted for publication and to list them in the monthly newsletter. More than 750 manuscripts were written by NIEHS scientists during the year. The Library published the 1987 NIEHS Bibliography of papers published by Institute personnel.

Experimental Data Repository: The Library continued the project of having laboratory notebooks microfilmed for archival purposes.

Library Interns: NIEHS, through an Interagency Agreement with EPA, obtained assistance from graduate students in the UNC School of Library Science. Three students worked half-time for the NIEHS Library as interns in the one-year appointments. They performed services in cataloging and serials management and managed the DBRA branch library.

Offices and Meetings: Dav Robertson maintained close contact with various library and information organizations in FY88. He served as Chairman, Public Relations Committee, of the N. C. Chapter of the Special Libraries Association (SLA), and he was chosen as President-elect of the chapter for 1988-1989. He served on the Advisory Council for the N.C. Library Staff Development Program centered at N.C. Central University. He also served on the Task Force on Ethical Issues for the N. C. Library Association. Mr. Robertson represented NIEHS at the national meeting of SLA in Denver and the quarterly chapter meetings in North Carolina. He also represented NIEHS at the C. L. Systems Users Group meetings in Portland, Maine, and in Philadelphia. Larry Wright served on the Board of Directors of the N. C. Chapter of SLA and helped plan the quarterly meetings. He also represented NIEHS at the national SLA meeting in Denver and was nominated as Chairman of the Biological Sciences Division. In addition, he served as Secretary for TRI-LIBS, the RTP Association of Information Specialists. To keep skills up to date, every staff member attended at least one training course or library association meeting during the year.

DIVISION OF INTRAMURAL RESEARCH

OFFFICE OF SCIENTIFIC DIRECTOR

DIVISION OF INTRAMURAL RESEARCH
Summary Statement

Scientists in the Division of Intramural Research (DIR) of the National Institute of Environmental Health Sciences (NIEHS) have the primary mission of investigating how living organisms, especially the human, react and adapt to a constantly changing and, in many respects, hostile environment. Considering the complexity of earth's environment and the additional potentially dangerous materials introduced into the environment by humans, it is not surprising that the mission of DIR requires an array of scientific disciplines encompassing nearly every field of biology and chemistry. Unique among the NIH intramural divisions, DIR has no clinical responsibilities; its purpose is not disease-oriented. On the other hand, to the extent that the environment poses risks to human health, DIR scientists develop the basic knowledge necessary for colleagues in other divisions of NIEHS to test and evaluate chemicals and other substances for their risk to human health.

Administratively, the Laboratories in DIR have been expanded to nine including the Comparative Medicine Branch (CMB). The new Laboratories are the Laboratory of Molecular Carcinogenesis (LMC) under the direction of Dr. Carl Barrett (formerly of the Laboratory of Pulmonary Pathobiology [LPP]), the Laboratory of Molecular and Integrative Neuroscience (LMIN) under the direction of Dr. Andres Negro-Vilar (formerly of the Laboratory of Reproductive and Developmental Toxicology [LRDT]), and the Laboratory of Cellular and Molecular Pharmacology (LCMP) under the direction of Dr. James W. Putney, Jr., (formerly Professor of Pharmacology at the Medical College of Virginia). The Laboratory of Genetics (LG) has been divided into two Laboratories. Dr. Burke Judd remains as Chief of LG whereas the new Laboratory of Molecular Genetics (LMG) has an Acting Chief, Dr. Charles Langley. The Laboratory of Molecular Biophysics (LMB) remains intact with Dr. Colin Chignell as its chief.

Brief summaries of some of the Division's major findings are as follows:

LMIN: Gonadal steroids enhance the secretion from the hypothalamus of the hormone (LHRH) that stimulates release of luteinizing hormone from the anterior pituitary. This unexpected finding has implications for the feed-back control of the functions of this important reproductive hormone. Opioid peptides (dynorphin, enkephalin) synthesized or released from the hippocampus appear to be related to the development of seizures. These studies provide additional means for understanding the etiology of epilepsy and the development of new and better pharmacological therapies.

LCMP: Although insulin is known to react with specific surface membrane receptors in cells, it was discovered that insulin directly infected in frog oocytes induces marked increases in protein and RNA synthesis. These effects are seen at concentrations below that required to observe effects of insulin at the cell surface. These findings have important implications regarding the site(s) of action of the hormone and may lead to a better understanding of the complex actions of this essential hormone.

LMB: Prostaglandin H synthase metabolizes a number biologically important compounds, including arachidonic acids and potentially toxic compounds found in foods. Enriched in the bladder, it metabolized the bladder carcinogen, 2-aminofluorene, to a chemical species that is probably responsible for the toxic effects. The enzyme converts arachidonic acid to prostaglandins that control chloride secretion in secretory glands involved in the disease cystic fibrosis. This discovery has important implications for understanding the genetic defect in this widely distributed disease.

LPP: Macrophages specific for pulmonary tissue express a variety of arachidonic metabolites including those that exert inflammatory effects on surrounding cells. They also secrete mitogenic factors or hormones, such as platelet-derived growth factor (or its homologues), that are known to induce proliferation of cells. Production and secretion of these substances is greatly enhanced in lung macrophages exposed to noxious particles such as asbestos and silica. It is likely that the macrophage-released factors are causal agents in the serious pulmonary disease afflicting humans exposed to these particles.

LRDT: It has been discovered that mouse spermatogenic cells are enriched with a protein (P70) that is homologous in structure to so-called heat-shock (or stress) proteins found in practically all living cells. Although the precise function of P70 remains unknown, it would appear that it is vital for germ differentiation and perhaps embryonic development. The importance of this discovery is that it may give a better understanding of the effects of environmental agents on male reproduction.

LMG: DNA polymerases are components of gene replication and comprise a family of enzymes that vary in their accuracy of DNA synthesis. Human and yeast DNA replication complexes have been found that are considerably more accurate in DNA replication than purified DNA polymerases from various sources, indicating that replication is subject to a more sophisticated process than hitherto considered. These are important findings for understanding the role of mutagenesis in environmentally-caused illnesses.

LMC: Concrete evidence for the existence of "suppressor" genes in animal cells has been given substantial credibility from recent studies using a novel gene transfer assay and a cDNA library prepared from normal human fibroblasts in a mammalian expression vector coupled with a highly efficient transfection procedure and a negative selection method. This and other detection procedures have advanced to the stage that makes it possible to finally delve into the structure and function of genes that control growth in animal cells.

CMB: Full accreditation was given to the Institute by AAALAC after formal inspection of NIEHS animal programs and facilities. Particular praise was given to CMB for its animal care and use programs, disease surveillance, methods for animal procurement, and general animal husbandry, a major achievement arising from the diligence and expertise of Dr. Herbert Amyx, Chief, CMB.

An unusual meeting with the Board of Scientific Counselors was held in January. In contrast to previous meetings, the Board was exposed to talks given by all of the permanent scientific staff to DIR over a two-day period. Although not comprehensive, this one-time, one-of-a-kind meeting had the merit of giving all of the new Board Members an opportunity of viewing the entire program and therefore of obtaining a better understanding of the complex mission of DIR. In general, the Board Members were very impressed with the scientific productivity.

A major outgrowth of the meeting was the preparation of a rather comprehensive description by each lab scientist of major accomplishments, publications, and where the projects may be leading.

COMPARATIVE MEDICINE BRANCH

COMPARATIVE MEDICINE BRANCH
Summary statement

The Comparative Medicine Branch (CMB), administratively located within the Division of Intramural Research, extends a broad range of services to all of the NIEHS research programs through five functional units: Animal Husbandry, Quality Assurance, Veterinary Medicine, Glassware and Media, and Office of the Chief. Major research animal responsibilities include program and facility management, health surveillance and diagnosis, procurement, clinical veterinary services, rodent breeding, technical assistance, quality assurance for food, bedding and water, and advice to the Institute on important animal issues. CMB also assists the scientific staff by providing glassware and media services, training for investigators and technicians, quality assurance support for research projects, consultation with investigators planning animal research projects, and administrative support to the Institute Animal Care and Use Committee. Independent and collaborative research studies relative to the major missions of the Branch are also conducted.

Two major outside reviews of the animal care and use program administered by CMB were conducted. The NIH Office of Animal Care and Use (OACU) selected NIEHS for the first in a series of program reviews designed to report to the Deputy Director of NIH the state of compliance of the BIDs with PHS Policy. Three representatives of OACU spent two days inspecting the animal facilities and reviewing program procedures and records. The NIEHS animal programs were complimented for leadership, organization, and attention to policy, and were proposed as a model program for the rest of NIH.

The American Association for Accreditation of Laboratory Animal Care, an independent, unaffiliated accrediting body also conducted its formal inspection of NIEHS animal programs and facilities. The AAALAC report continued full accreditation for the Institute, commending CMB for an animal care and use program of high quality. Selected for particular praise were the programs for disease surveillance, animal procurement, and general animal husbandry. No deficiencies were noted in the report and no suggestions for correction or improvement of the program were deemed necessary.

Several major personnel changes have occurred. Dr. Diane Blackmore has joined CMB as Clinical Veterinarian, transferring from the National Institute of Mental Health. Dr. Blackmore has already expanded clinical assistance to research studies and is strengthening our overall animal health program. Mr. Clinton Robert Moorman was recruited to fill the Animal Husbandry Supervisor vacancy created when Ms. Barbara Sawyer left CMB to attend medical school. Mr. Moorman has extensive experience in toxicology support, an excellent academic background, and certification as an AALAS technologist.

In collaboration with the Gamete Biology Section of the Laboratory of Reproductive and Developmental Toxicology, CMB has established a transgenic mouse resource for the Institute. Initial studies using the transgenic technology will focus on gene regulation of development and the regulation of oncogenes. The studies will use the B6C3F1 hybrid and the newly obtained FVB/N strains of mice.

A major contract for animal facility operation at the North Campus and service areas operation for the South Campus has been completed and a Request for Proposals has been issued. Conversion of these areas to contractor operation i scheduled for October 1988. A contract has also been awarded for the development of an informational brochure to illustrate the benefits to human health that have resulted from animal research at NIEHS.

The Glassware section has undergone successful conversion to contractor operation. The contractor, Transcontinental Enterprises, has compiled an outstanding record of consistent, quality service to the Institute. Government employee relocation was smooth and uneventful. One employee chose to work for the contractor, two retired, and the remainder were relocated within CMB to the Media section and the Animal Husbandry section.

The Media section has completed conversion of all media recipe data files for Institute investigators to computerized storage and retrieval. All ordering of supplies for both Media and Glassware sections is now also computer assisted. In the month of April, Media recorded a perfect score for quality control compliance, the first time this has been achieved. Given the quantity and variety of medias prepared for the Institute, this was a remarkable accomplishment and an important milestone in the quality assurance program for the Media section.

The Quality Assurance Lab (QAL) has performed 30,378 tests on 6,144 cases from April 1987 to March 1988. In addition to managing animal health surveillance, the testing of food, water, and bedding, and quality control for glassware and media, the QAL also provides pest control support to all NIEHS buildings, the cafeterias and child care centers, and the animal facilities. The QAL receives an average of one pest control problem call per day. Mr. Gordon Caviness, a certified pest control specialist, was added to the staff in December 1987. The QAL, as consultants for the use of herbicides at the Institute, has reevaluated NIEHS pesticide policies and recommended changes in procedures.

Animal health programs designed to eradicate Sendai virus infections and pinworms in rats on the North Campus in the spring and summer of 1987 have proven to be effective. The animal populations have remained free of these agents for the past year. The South campus animal facilities have remained free of known pathogenic bacteria, mycoplasma, adventitious murine viruses, ectoparasites and pinworms since January 1987.

As part of our research effort into the natural history of Mouse Hepatitis Virus (MHV), a study was undertaken to identify latent foci of natural infection in an animal room on the North Campus. This room was suspected of harboring MHV because of past outbreaks, but had been negative for MHV for the previous year using conventional sentinel animal methods. Using a new sentinel animal system employing sensitive strains of mice housed on bedding taken from experimental animals, we were able to identify a low level focus of carrier animals and take measures to permanently eliminate the virus from this colony. This project also offered the opportunity to compare two sentinel animal detection systems side by side under conditions which test the sensitivity of the methods.

In addition to studies on the natural history of Mouse Hepatitis Virus, CMB also conducted research on the natural history and transmission of Pneumonia Virus of Mice in euthymic and athymic mice. Studies were undertaken to compare means and duration of virus transmission using several strains of PVM in three strains of mice. Preliminary results indicate that ATCC VR-25, Strain No. 15, is not easily transmitted between mice and that the strain of PVM could be an important factor in predicting the spread of a natural outbreak.

The next year will see major changes within CMB. As the contract goes into effect for the North Campus animal facility, government employees from this area will be relocated to the South Campus to open and maintain the E module animal facility. This will alleviate a tight housing situation in C & D modules, allow expansion of in-house breeding programs, and provide investigators with badly needed hazardous study animal rooms. CMB technical assistance duties have continued to grow, and will expand further as E module is occupied. These changes should facilitate the mission of CMB to assist and enhance basic research at NIEHS through its animal programs and facilities.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 22103-05 CMB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Natural History of Mouse Hepatitis Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J. E. Thigpen	Head, Quality Assurance Lab	CMB, NIEHS
Others:	D. E. Blackmore	Vet. Med. Officer	CMB, NIEHS
	E. H. Lebetkin	Biol. Lab. Tech., QAL	CMB, NIEHS
	M. L. Dawes	Biol. Lab. Tech., QAL	CMB, NIEHS
	G. F. Caviness	Biol. Lab. Tech., QAL	CMB, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Comparative Medicine Branch

SECTION

Quality Assurance Laboratory

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.3	.1	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A conventional CD-1 mouse colony, which was suspected to harbor low incidence levels of MHV and mites, was used to compare two different exposure routes for sentinel animals. For the last eight months sentinel mice, not exposed to dirty bedding, were negative for mites and MHV. The objectives of this study were to compare two different sentinel animal exposure routes and to determine whether these agents were present in this colony. Eight cages of experimental sentinel mice, containing 12 mice/cage, housed without filter bonnets, were placed in the room on four different racks. Twice each week, four cages received a composite sample of bedding used previously by mice in the room. The remaining four cages received fresh unused bedding. Sentinel mice were monitored at monthly intervals. After four months, mice from two cages which received used bedding seroconverted to MHV and one cage was positive for mites. Three weeks later, all four cages of mice which received used bedding were positive for MHV and three were positive for mites. In contrast, only two of four cages of mice which received fresh bedding were positive for MHV and all were negative for mites. These findings indicate the importance of exposing sentinel mice to used bedding and that MHV and mites may go undetected for several months in a mouse colony when the incidence levels are less than 10% and where strict sanitation practices are used.

It was concluded that MHV and mites are more easily transmitted to sentinel mice by exposing sentinel mice to dirty bedding. As a result of these findings, all sentinel animals are exposed to dirty bedding and we have improved our sentinel animal program.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 22107-03

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Natural History of PVM

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J. E. Thigpen	Head, Quality Assurance Lab.	CMB, NIEHS
Others:	D. E. Blackmore	Vet. Med. Officer	CMB, NIEHS
	E. H. Lebetkin	Biol. Lab. Tech., QAL	CMB, NIEHS
	M. L. Dawes	Biol. Lab. Tech., QAL	CMB, NIEHS
	G. F. Caviness	Biol. Lab. Tech., QAL	CMB, NIEHS

COOPERATING UNITS *(if any)*
Div. of Lab Animal Med., Duke University (C. B. Richter)

LAB/BRANCH
Comparative Medicine Branch

SECTION
Quality Assurance Laboratory

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.3	.2	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Pneumonia virus of mice (PVM) infects mice, rats, hamsters and guinea pigs. Because of the broad spectrum of rodent hosts, PVM has the potential to influence many experimental results, notably those studies involving the cell dynamics of pulmonary parenchyma. The objective of this project is to study the natural history and transmission of PVM in euthymic and athymic mice. A supply of stock PVM virus (ATCC VR-25, Strain No. 15) has been produced in BHK-21 cells and in nude mice (10% lung suspension) for future studies. Studies were undertaken to determine means and duration of virus transmission from experimentally infected CD-1, C3H/Hen and nu/nu mice to susceptible cage mates (added at specified intervals post infection) and to other mice housed in adjoining cages with or without filter bonnets. Mice were infected by the nasal route with 0.04 ml of the appropriate virus suspension. Preliminary results indicate that mortality rates in the CD-1 mouse differ from those of the C3H/Hen and nude mouse when infected with the ATCC strain of PVM virus. Preliminary results also indicate that the ATCC strain of PVM is not readily transmitted to cage mates even after three passages in the nude mouse. None of the mice housed in adjacent cages, with or without filter bonnets, seroconverted to PVM.

These preliminary results indicate that PVM is not easily transmitted among mice. A stock supply of a wild or street strain of PVM isolated from naturally infected nude mice will be produced for comparative studies with the ATCC strain.

LABORATORY OF CELLULAR AND MOLECULAR PHARMACOLOGY

LABORATORY OF CELLULAR AND MOLECULAR PHARMACOLOGY
Summary Statement

The Laboratory of Cellular and Molecular Pharmacology at NIEHS is interested in the mechanisms that govern toxic reactions brought about by exposure to harmful chemicals present in the environment. Because many biological systems are not sufficiently understood to allow for mechanistic conclusions to be drawn from results of experiments involving the direct use of toxic agents, our emphasis is placed upon basic research aimed at defining certain biological systems at the molecular level, with particular attention being paid to regulatory mechanisms.

One element in common to all of the research programs in The Laboratory of Cellular and Molecular Pharmacology is membrane function. This is an integral aspect of our studies on the metabolism of environmental chemicals by membrane-bound enzymes, the transport of solutes across epithelial membranes and the modification of cellular responses by surface membrane receptors. Within all three areas of investigation, the first goal is to understand the operation of the systems at the molecular level. Once this has been accomplished in sufficient detail, it should be possible to determine the mechanisms by which various toxicants perturb these systems, and to enumerate the implications of such interactions on human health.

Many harmful substances present in the environment exhibit little or no biological activity unless they first undergo some chemical modification. For the most part, this is accomplished by oxidative reactions that are catalyzed by enzymes present in most mammalian tissues. Although it is clear that this biotransformation is a prerequisite to most chemically induced carcinogenic, mutagenic, and other manifestations of toxicity, the role metabolism plays in directing the tissue- and cell-specificities of these events is not understood. This particular problem is being assessed by investigating drug-metabolizing enzymes in liver and lung, two tissues that respond quite differently to a number of toxic chemicals. The primary enzymes involved with the metabolism of toxic chemicals are various isozymes of cytochrome P-450, and the distribution, regulation, and concentrations of these isozymes in liver and lung have been found to be markedly different. At the molecular level, studies using recombinant DNA technology have detected more subtle distinctions in different molecular forms of these isozymes. In some instances these differences appear to be important factors in site-directed toxicity. For example, the pulmonary toxicity of certain substituted furans, which exert their effects on a specific population of pneumocytes, appears to result from the relatively high concentrations of two isozymes of cytochrome P-450 present in the target cell type. These two isozymes are also present in liver but at much lower concentrations.

Transport of chemicals across membranes is another controlling factor in the biological disposition of environmental chemicals. This process is associated with uptake into the organism, distribution within the organism, and excretion. In addition, the transport of biological molecules across membranes, which is part of the normal metabolic process, can be drastically altered by a variety of environmental compounds. Because of their complex organization, functional importance, and exposed locations, epithelial membranes are particularly susceptible to such toxic effects. This has important implications with respect to the transport functions of the kidney, which controls, via excretion, the time of exposure to toxic chemicals. It is now known that the process of excretion of organic anions requires the actions of two carrier proteins. One of these proteins exchanges internal glutaric acid for the molecule to be excreted and the second utilizes the sodium gradient to maintain a high intracellular concentration of glutarate. A second transport mechanism for organic cations has also been found to be carrier-mediated. This process is strongly potential dependent, such that the major driving force responsible for the intracellular accumulation of organic cations is the electrical potential gradient across the cell's basolateral membrane.

Surface-membrane receptors are central to the processes by which cells receive the information required for the regulation of their functions. In order to maintain proper biochemical balances, these receptors, and the intracellular reactions they control, must operate with a high degree of precision. Because these systems are highly complex, they offer numerous opportunities for their actions to be modified by various environmental toxins or other injuries. For example, such systems are involved in the actions of numerous growth factors, and it is now clear that many oncogene products represent altered components of growth factor signal transduction mechanisms. The implications of this are just beginning to be understood, but should become clearer when cellular signal transduction is better understood.

The intracellular concentration of calcium plays a central role in the integration of cell metabolism, and the regulation of cellular calcium is an area of increasing study in The Laboratory of Cellular and Molecular Pharmacology. Two major mechanisms are involved in the mobilization of calcium as a cellular signal. One involves the generation of messenger molecules called inositol phosphates. The pathways involved in the metabolism of inositol phosphates are being investigated, and the elucidation of several novel reactions has uncovered new metabolites that may play a major role in the control of calcium. Recent findings indicate that an early event in the action of certain receptors is the hydrolysis of a membrane phospholipid with the subsequent formation of two putative second messenger molecules, diacylglycerol and inositol 1,4,5-trisphosphate. The first of these is thought to activate a specific protein kinase, the second is thought to be involved in the release of calcium from intracellular stores. The trisphosphate compound is further metabolized by two distinct pathways resulting in a complex mixture of inositol phosphates all of which may have additional biological functions. The formation of inositol phosphates is controlled by peptide receptors located on the cell surface. One of these receptors, which appears to be regulated by desensitization, is being studied in detail.

A second mechanism for regulation of cellular calcium is through voltage-activated calcium channels in the surface membrane. By using the powerful patch-clamp methodology, the regulation of these channels by calcium and by other hormone-derived messengers is being investigated on individual calcium channels in cell-free patches of native membrane. With this technique, it has been possible to demonstrate directly that phosphorylation by specific kinases is necessary for the channel respond normally to changes in membrane potential. This phosphorylation dependence of channel activity and its removal by endogenous calcium-activated phosphatases provide a simple framework for understanding the regulation of calcium fluxes across the plasma membrane in response to neurotransmitters as well as the abnormal changes that result due to the toxic actions of various xenobiotic agents.

In addition to receptors on the cell surface, recent findings indicate that there are also intracellular receptors for some compounds. Marked increases in both protein and RNA synthesis have been observed in frog oocytes following direct injection of insulin at concentrations below those required to initiate processes at the cell surface. The unique aspect of this work is the development of techniques that allow for changes to be monitored in single isolated cells. The microinjection system enables the researcher to bypass the cell membrane and observe the direct effects of substances in the cell. Results to date indicate that insulin, which is known to mediate cell function at least in part by interacting with a surface receptor, has a direct effect that does not require binding to molecules on the outside of the plasma membrane. Furthermore, the effects of intracellular insulin, presumably acting on intracellular receptors, appears to involve a mechanism which is distinct from that involving the initial binding of insulin to its surface membrane receptor.

While the study of the metabolism and membrane transport of foreign chemicals in The Laboratory of Cellular and Molecular Pharmacology represents a continuation of investigations that were initiated some time back, the work on signal transduction was initiated just over one year ago and was discussed for the first time in the preceding Annual Report. This important area of research, which has not been a major part of the effort at NIEHS in the past, will be expanded in the future. The importance of receptor mechanisms to environmental health may not as yet be understood, but the central role these systems play in the maintenance of biological functions indicates that they are a likely target for toxic insult. Also, the direct interaction of some toxicants, such as TCDD, with receptors has been clearly established. However, our lack of understanding of the molecular events that occur subsequent to receptor-ligand interaction, has blocked all efforts to define the mechanisms by which chemicals like TCDD exert their effects. In addition, the role of cellular calcium metabolism in the toxic actions of a wide variety of xenobiotics (including carcinogens and transforming oncogenes) is now well established. Clearly, the molecular pathways that transmit messages between the cell surface and the intracellular metabolic machinery must first be unraveled before we will be in a position to define how signal transduction can be affected by environmental toxins and other xenobiotics.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 80001-16 LCMP

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Microsomal Mixed-Function Oxidase System: Structure and Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R.M. Philpot	Research Chemist	LCMP	NIEHS
Others:	R. Gasser	Visiting Associate	LCMP	NIEHS
	D. Duignan	IRTA Fellow	LCMP	NIEHS
	J. Schulze	Visiting Fellow	LCMP	NIEHS

COOPERATING UNITS (if any)
University of California, Davis, CA; Scripps Institute and Research Foundation; North Carolina State University; University of Maryland

LAB/BRANCH
Laboratory of Cellular and Molecular Pharmacology

SECTION
Molecular and Comparative Pharmacology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
6.0	4.0	2.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Rabbit cytochrome P-450 isozymes 2 and 5 are present in lung and liver. In the liver, but not in the lung, the concentrations of these isozymes are increased by treatment of rabbits with phenobarbital. Homologues of isozymes 2 and 5 have been detected in lungs of mice, rats, hamsters, guinea pigs, and monkeys. Although homologues of isozyme 2 are also present in livers of these species, hepatic homologues of isozyme 5 are not detected in any species except the hamster. Treatment of hamsters with phenobarbital does not increase the hepatic concentration of the isozyme 5 homologue. The primary structure of isozyme 5 has been derived from the nucleotide sequences of cDNAs. Independent cDNA clones have been obtained from mRNA of lung, liver and kidney of rabbit. In all three cases, identical sequences have been obtained. No evidence of multiple forms of isozyme 5 has been obtained either from cDNA libraries or analysis of genomic DNA. The sequence of the rat homologue of isozyme 5 has also been derived and found to be highly related to the rabbit enzyme. In fact, the extent of the similarity between this isozyme from rat and rabbit (about 90%) is much greater than the similarity between isozyme 2 from these species. In the case of isozyme 2, multiple forms have been defined. At least three forms of this isozyme can be found in rabbit liver depending upon the phenotype of the animal. Two of these forms appear to be allelic varients that are expressed in both liver and lung. The third form is found only in liver. Preliminary results indicate that an additional form is expressed in rabbit kidney. The concentration of the form in kidney is markedly increased following treatment of rabbits with phenobarbital. Phenobarbital also appears to increase the renal content of isozyme 5. Rabbit isozyme 5 and the homologue in rats are related to a form of P-450 in rats that hydroxylates lauric acid. The equivalent form in rabbits hydroxylates prostagladins. It is of interest that isozyme 5 is inactive with prostaglandin substrates but is highly efficient in the hydroxylation of lauric acid.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80007-16 LCMP

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Conjugation and Oxidation Pathways for Xenobiotic Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Cosette J. Serabjit-Singh Research Chemist LCMP NIEHS

The project has been terminated as the lab is no longer in operation.

COOPERATING UNITS *(if any)*

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80031-12 LCMP

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Role of Altered Membrane Function in Xenobiotic Toxicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.B. Pritchard	Research Physiologist	LCMP	NIEHS
Other:	D.S. Miller	Expert	LCMP	NIEHS
	P.M. Smith	Visiting Fellow	LCMP	NIEHS

COOPERATING UNITS (if any)
University of Florida; Duke University

LAB/BRANCH
Laboratory of Cellular and Molecular Pharmacology

SECTION
Molecular and Comparative Pharmacology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
7.6	3.0	4.6

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The ability to transport solutes across epithelial membranes is a vital function of many organs, e.g., kidney, choroid plexus, gut. In turn, epithelial transport depends upon individual transport systems located in apical (BBM) and basolateral (BLM) membranes. Because of their complex organization, functional importance, and exposed location, epithelial membranes are particularly susceptible to toxic effects of foreign chemicals. Major recent emphasis has focused on the renal organic anion (OA) transport system, which determines the extent of elimination of many toxic xenobiotics. Isolated membrane vesicle studies demonstrate that OA transport is driven by indirect coupling to the Na gradient. An anion exchanger mediates exchange of external OA for internal glutaric acid (or a-ketoglutarate) and a second carrier taps energy stored in the Na gradient to drive glutarate back into the cell, maintaining the steep (in>out) glutarate gradient needed to drive OA uptake. Thus, BLM uptake leads to intracellular accumulation. Intracellular OA then exits across the BBM via another anion exchanger, producing net tubular secretion. Recent whole tissue studies not only confirm the importance of this system in rat kidney, but also demonstrate its contribution to OA transport in the kidneys of several other species and in other tissues, including the choroid plexus. Similar studies utilizing both electrophysiological and radiochemical techniques to examine the organic cagtion (OC) transport system, show that the basolateral step in OC secretion is carrier-mediated and derives its primary driving force from the membrane potential. Membranes also play important roles in information transfer between the cell and its environment. Amphibian oocytes were used to examine the nature of insulin in action. These studies demonstrate that insulin, at physiological doses, acts directly on both membrane and intracellular sites to alter protein and RNA synthesis. Effects of intracellular and extracellular insulin were additive, suggesting separate modes of action.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80042-02 LCMP

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Calcium Regulation and Signal Transduction Mechanisms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	James W. Putney, Jr.	Chief	LCMP	NIEHS
Others:	Arlene Hughes	Staff Fellow	LCMP	NIEHS
	Hiroshi Sugiya	Visiting Associate	LCMP	NIEHS
	Frank Mennitti	Staff Fellow	LCMP	NIEHS
	Kerry Oliver	Staff Fellow	LCMP	NIEHS
	Haruo Takemura	Visiting Fellow	LCMP	NIEHS
	Gary Bird	Visiting Fellow	LCMP	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Cellular and Molecular Pharmacology

SECTION
Calcium Regulation Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.0	6.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The broad aim of this project is to understand at the cellular and molecular level the mechanisms by which surface membrane receptors for hormones, neurotransmitters and growth factors modify cellular responses through mobilization of cellular Ca^{2+}. An early event in the action of receptors of this class is the hydrolysis of a membrane phospholipid, phosphatidylinositol 4,5-bisphosphate to yield two putative second messengers, diacylglycerol (DG) and inositol, 1,4,5-trisphosphate [$(1,4,5)IP_3$]. DG activates a specific kinase in cells, designated protein kinase C, and $(1,4,5)IP_3$ releases Ca^{2+} from an intracellular store, presumably a component of the endoplasmic reticulum. Recently, a number of novel inositol polyphosphates have been discovered, including $(1,3,4)IP_3$, $(1,3,4,5)IP_4$ and (cyclic $1{:}2,4,5)IP_3$. Each of these has been shown to have biological activity in *in vitro* assay systems. These studies will attempt to combine *in vivo* (cells) assays for the amounts and kinetics of these and other potentially important inositol phosphates with real time measurements of cytosolic Ca^{2+} using ratioing Ca^{2+} indicators, such as fura-2, and with *in vitro* assays of their actions on subcellular Ca^{2+} metabolism, in order to estimate the relative contributions of these various species to cellular Ca^{2+} signalling mechanisms. In a related project, the mechanisms by which the phospholipase C-linked receptors are regulated is being investigated, using the parotid substance P receptor as a model. This receptor appears to be regulated by both heterologous and homologous mechanisms, with the latter being the quantitatively more important. The heterologous mechanism involves feed back inhibition at a step subsequent to receptor activation by the protein kinase C. Since Ca^{2+} is believed to play a central role in mechanisms of chemically-induced cell injury, these studies should provide insights into the mechanisms underlying the pathophysiological consequences of exposure to toxins and other environmental agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80043-01 LCMP

PERIOD COVERED
October 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Ion Channel Modulation by Signal Transduction Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	David L. Armstrong	Senior Staff Fellow	LCMP	NIEHS
Others:	Marietta Austin	Biologist	LCMP	NIEHS
	Luca Turin	Visiting Scientist	LCMP	NIEHS

COOPERATING UNITS (if any)
Eckert Laboratory, Department of Biology, University of California at Los Angeles, Los Angeles, California

LAB/BRANCH
Laboratory of Cellular and Molecular Pharmacology

SECTION
Membrane Biology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.75	1.25	0.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

By controlling the voltage across the plasma membrane, ion channels regulate a number of important cellular processes. Recent advances in electrophysiological techniques allow single ion channels to be studied under voltage-clamp in cell-free patches of native membrane. We are using this patch-clamp technique to investigate the molecular mechanisms through which ion channel activity can be modulated by the intracellular biochemical pathways that transduce extracellular signals into altered cellular responses. Initially, we have focused on the dihydropyridine-sensitive calcium channel that is activated by membrane depolarization in mammalian tumor cell lines. We have obtained the first direct evidence that these channels must be phosphorylated by the cyclic AMP-dependent protein kinase in order to respond to membrane depolarization. We have also demonstrated that, unlike other voltage-activated ion channels which inactivate during sustained depolarization, dihydropyridine-sensitive calcium channels only inactivate when channel activation leads to calcium ion entry and accumulation inside the cell. Our most recent experiments suggest that calcium promotes inactivation by stimulating an endogenous protein phosphatase to dephosphorylate the channel protein. These results provide a simple framework for understanding calcium channel regulation by extracellular signals, clinically important drugs, and environmental toxins.

LABORATORY OF GENETICS

LABORATORY OF GENETICS
Summary Statement

The Laboratory of Genetics went through a major reorganization in September 1987. Nine key researchers and their work groups were removed from the Laboratory. There are now three work groups, comprised of seven full time permanent employees, two visiting fellows and a student. The focus of the Laboratory has changed somewhat and the scope has been narrowed considerably. The remaining projects include studies of gene expression and regulation in mammals by Dr. Steven Li, the analysis of mouse skeletal abnormalities in offspring from mutagen treated parents by Dr. F. M. Johnson and the development of stably integrated vectors in mammalian cells as a tool for the *in vivo* study of mutagenesis by Dr. Heinrich Malling.

More specifically, Li is studying the molecular organization and expression of mammalian lactate dehydrogenase genes and the structure of prostatic acid phosphatase isozymes. Full length cDNAs for human LDH-A and LDH-B as well as mouse LDH-C isozymes have been cloned and expressed in *E. coli*. The properties of these enzymes and their multiple functions are being examined and the tissue and stage specific regulation of their expression is being determined. Of particular interest are observations that the LDH isozymes assume a variety of roles in mammalian cell structure and function beyond their roles as dehydrogenases. Other labs have reported that the rat liver single-strand DNA binding protein is LDH-A4, that the ϵ-crystalline of duck lens is LDH-B_4 and that human centrosomal proteins possess a common epitope with LDH-B4. Li is examining the structural aspects of the proteins relative to functional domains involved with these several roles. He is also actively investigating the mechanisms of expression and regulation, particularly in regard to LDH-C which is expressed in cell specific fashion in the testis.

Li is also studying human acid phosphatase genes relative to the regulation of expression in prostate gland differentiation. Several human prostate acid phosphatase (PAP) cDNA clones have been isolated and the nucleotide sequences are being determined. Amino-terminal sequences of PAP-1 and related glycoprotein have been determined. Partial amino acid sequences of peptides from CNBr and protease cleavage of PAP-1 have been determined and this information is being correlated with the nucleotide sequence of the cDNAs. This investigation could lead to the development of better diagnostic markers than now exist for detection of human prostate cancer.

The Johnson group is studying methods for detecting natural and induced mutations in the mouse and is investigating various aspects of the mechanisms of mutation induction. Most recent work involves the morphometric analysis of mouse skeletons of offspring from mutagenized fathers in an attempt to develop tools to investigate genetic risk from mutagen exposure. Most skeletal variation is too slight to be noticed by visual inspection, however, high resolution morphometric analysis has detected significant skeletal differences, as compared to control groups, in a large proportion of animals in the brood produced 12 weeks following ethylnitrosourea treatment. Refinement of the methods for measurement and analysis will allow critical evaluation of this system for use in risk assessment studies.

The Malling group has focused on development of systems to study mammalian mutagenesis *in vivo*. One system uses ϕX174 integrated into chromosomes of mouse L cells. Techniques have been developed for recovery and analysis of the ϕXDNA following mutagen treatment of transformed cells. Viable phage are produced in sufficient numbers to study mutation induction and to characterize mutation events at the DNA level.

Dr. Malling has spent a sabbatical year in Professor Alan Fersht's lab at London Imperial College of Science and Technology. As an outgrowth of the work done there, development of shuttle vectors to be placed in transgenic mice is now underway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 60099-09 LG

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Organization-regulation of mammalian lactate dehydrogenase genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Steven S.-L. Li	Research Geneticist	LG, NIEHS
Others:	B. Yukihiro Hiraoka	Visiting Fellow	LG, NIEHS
	Tetsuo Takano	Visiting Fellow	LG, NIEHS
	Esther W. Hou	Biologist	LG, NIEHS
	Farida S. Sharief	Biologist	LG, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.4	2.5	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The cDNAs for human LDH-A and -B, and mouse LDH-A, -B and -C isozymes have been cloned and sequenced. Human LDH-A, -B and -C genes and pseudogenes have been mapped and isolated. The genomic structure of human LDH-A -B and -C genes as well as mouse LDH-A gene have been characterized. The protein-coding sequences of mammalian LDH-A, -B and - C genes are interrupted by six introns, and their relationships between protein structure and exon organization are illustrated. The developmental and tissue-specific expression of mouse LDH-A, -B and -C genes have been studied. The expression of mouse LDH-A gene was induced by cyclic AMP and estrogen. This information will allow more accurate evaluation of genetic mutation events caused by mutagens and eventually will be of value to improve human health care.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61019-08 LG

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Collaborative protein sequencing and peptide synthesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Steven S.-L. Li	Research Geneticist	LG, NIEHS
Others:	Farida S. Sharief	Biologist	LG, NIEHS

COOPERATING UNITS *(if any)*
Department of Chemistry, University of North Carolina, Chapel Hill, North Carolina

LAB/BRANCH
Laboratory of Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The isolated human prostatic acid phosphatase isozymes was subfragmented by CNBr and Lys-C protease. The partial sequences of the purified peptides were determined, and, thus far, a total of 120 amino acids, approximately 25% of the protein residues, have been sequenced. This information is being used for the cDNA cloning. The collaborative protein chemistry laboratory with UNC has already provided lots of research services on protein microsequencing and peptide synthesis to other scientists at the NIEHS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 61032-05 LG

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure-function of mammalian lactate dehydrogenase isozymes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Steven S.-L. Li	Research Geneticist	LG, NIEHS
Others:	Farida S. Sharief	Biologist	LG, NIEHS
	Jun M. Versola	Biological Aid (SIS)	LG, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.3	1.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The full-length cDNAs for human LDH-A, human LDH-B and mouse LDH-C isozymes have been expressed in E. coli. The enzymatic and non-enzymatic properties are being characterized. The cloned mouse LDH-C cDNA was inserted immediately downstream to the MMTV-5'LTR promoter, and it was shown to synthesize LDH-C polypeptide in Chinese hamster ovary cells. The mouse LDH-C subunit and the endogeneous Chinese hamster LDH-A subunit formed in vitro a heterotetrameric LDH-A3C1 isozyme, and this novel isozyme exhibited enzyme activity utilizing lactate as substrate. Spermatogenic cells isolated from adult and prepubertal mice were used to examine enzyme activities and synthesis of the lactate dehydrogenase isozymes during spermatogenesis. The synthesis and activity of LDH-C4 were detected earliest in preleptotene and lepotene/zygotene spermatocytes, and were prominent in pachytene spermatocytes. Somatic-type LDH isozymes consisting primarily of LDH-B subunits were present in germ cells throughout spermatogenesis. Sertoli cells were further shown to exhibit comparable amounts of five tetrameric LDH isozymes formed by combination of LDH-A and LDH-B subunits.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65021-16 LG

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Investigation of Germinal Mutation Induction in Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	F. M. Johnson	Research Geneticist	LG, NIEHS
Others:	M. L. Snell	Biologist	LG, NIEHS

COOPERATING UNITS *(if any)*
Dr. S. E. Lewis, Research Triangle Institute, Life Sciences Group, Research Triangle Park, N.C.; Dr. D. P. Lovell, British Industrial Biological Research Association, Carshalton, Surrey, England

LAB/BRANCH
Laboratory of Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are (1) to detect natural and induced mutations in mice, (2) to achieve understanding of the molecular events occurring in the process of mutation induction, and (3) to relate these events to the life, form and function of the mammalian organism, including man. Our project is relevant to the problem of human exposures to environmental chemicals, including mutagens and carcinogens, and to the increased risk of genetic disease that the offspring of exposed individuals may incur. We have detected many mutations at specific biochemical loci with electrophoretic methods, characterized several normal and mutant genes (and gene products), and examined the offspring of mutagen-treated and control parents for a physical manifestation of polygenic mutations affecting the skeleton. Recently, we developed an expanded set of skeletal characters and an independent method for evaluating metrical variation using X,Y coordinate data obtained using enlarged bone images projected on a digiting tablet. Applying these newly acquired means, we recently completed analysis of 1030 progeny from treated and control DBA/2J x C57BL/6J parents and discovered one group with a very highly statistically significant increase in variability. Much of this variation is too slight to be noticed by ordinary visual inspection, but a large proportion of animals were affected in the one group. These results suggest that morphometrical analysis may provide a more powerful tool to investigate problems of genetic risk than was previously believed. The results also suggest that these methods may be much more efficient and relevant than any available specific-locus test.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65033-05 LG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

In Vivo Mammalian Mutagenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	H. V. Malling	Research Geneticist	LG, NIEHS
	J. G. Burkhart	Research Chemist	LG, NIEHS

COOPERATING UNITS *(if any)*

C. A. Hutchinson, III & M. H. Edgell, UNC, Chapel Hill, N. C.
E. J. Eisen, NCSU, Raleigh, N. C.
A. Fersht, Imperial College, London

LAB/BRANCH

Laboratory of Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this research is to study chemical mutagenesis at the DNA level in mammals and to evaluate genetic and biochemical events in certain mutants as models of human genetic diseases. A major problem in mutagenesis is that the level and specificity of response is very different between indicator organisms; predictions about induced mutagenesis may not be relevant. Significant variation is due to the diversity of the marker genes; a single sequence needs to be used as a target in the various organisms and tissues. Our analysis is based on variance among single copies of the ϕX174 virus containing am3 and cs70 mutations as a shuttle vector in different species. The accomplishments are as follows. 1) Techniques have been developed to recover chromosomally integrated ϕX DNA and produce sufficient viable phages for the study of mutagenesis in cultures or tissues of transgenic mice. 2) The reversion frequency of am3 has been measured in progeny phage rescued from untreated mouse L-cells (5×10^{-7}) and cells treated with 20mM EMS (1.16×10^{-5}). 3) New cell lines containing ϕX have been produced. 4) Experiments are in progress to expand the indicator region of the ϕX phage to provide detection of many more mutations in the integrated phage DNA. The use of integrated viral vector in transgenic mice can combine a theoretical study of mechanisms of mutation in several model organisms with an assessment of mutagenic hazard. A single DNA sequence can be exposed and analyzed as naked DNA, as a single stranded virus, double stranded in bacteria, and as vector DNA in the nuclear genome of mammalian cells or transgenic mice. In addition, we have characterized a new genetic mouse biochemical model of transient neonatal jaundice as a unique UDP-glucuronyl transferase deficiency. Adults homozygous for the mutation appear phenotypically normal in terms of the jaundice but remain enzymatically deficient throughout life. The enzymatic deficiency is accompanied by generalized necrosis and liver enlargement in older mutants. Work is in progress to determine whether the mutation is in the structural gene or perhaps regulatory.

PHS 6040 (Rev 1/84) GPO 914-918

LABORATORY OF MOLECULAR BIOPHYSICS

LABORATORY OF MOLECULAR BIOPHYSICS
SUMMARY STATEMENT

Research efforts in the Laboratory of Molecular Biophysics are directed towards understanding at the molecular level the mechanisms of toxicity of environmental chemicals and the development of techniques to detect, analyze and quantitate extremely low levels of toxic agents in biological tissues. The studies draw mainly on the research disciplines of physics, physical chemistry, organic chemistry, pharmacology, biochemistry and cell biology. Special emphasis is placed on the following (1) the development, improvement and utilization of spectroscopic methods (nuclear magnetic resonance, electron spin resonance, fluorescence spectroscopy) to characterize and measure the molecular interactions that occur between environmental agents and target biological systems; (2) the conduct of physical organic and bioorganic studies of environmental agents, biological materials and their metabolic conversion products with emphasis on elucidation of chemical mechanisms in biological damage; (3) the development, improvement and utilization of analytical methodology (mass spectrometry, chromatography) for specified agents present in trace amounts; (4) the provision of collaborative and service functions (mass spectrometry, nuclear magnetic resonance) for other NIEHS laboratory and research programs.

The Laboratory is organized into five workgroups:

Molecular Biophysics: Research is concerned with understanding at the molecular level the interaction of chemical and physical agents with target biological systems including nucleic acids, proteins and membranes.

Mass Spectrometry: The work of this group involves the application of advanced mass spectrometric methods to solve analytical problems and the development of new techniques in anticipation of future analytical requirements.

Nuclear Magnetic Resonance (NMR): The objective of the NMR Program is to elucidate the mechanisms by which chemicals and heavy metals in the environment cause cell injury with the aid of advanced NMR techniques including magnetic resonance imaging (MRI) and *in vivo* spectroscopy.

Prostaglandin Biochemistry: This group investigates the metabolism of arachidonic acid to prostaglandins (PG), hydroxy fatty acids and leukotrienes and studies their role in a number of important physiological and pathophysiological events. The group also studies the metabolism of chemicals by prostaglandin synthase (co-oxidation) in order to determine the role of this metabolic pathway in the development of chemically-induced carcinogensis.

Metabolism: The Metabolism work group studies the manner in which environmental chemicals are absorbed into the body, accumulate in specific organs, are metabolically altered to other chemicals, and eventually are secreted and eliminated. The alteration products are identified, and studied to determine whether they or the original environmental chemicals are actually responsible for toxic effects. In addition, the group investigates the manner in which environmental chemicals interfere with normal metabolic processes occurring (primarily) in the liver.

Recent Scientific Accomplishments

Molecular Biophysics:

Free radicals are chemically reactive species that are thought to be responsible for the toxicity of many environmental agents. While evidence for the generation of free radicals is often easy to obtain in the test tube it is much more difficult to prove the existence of these species in the intact animal, or in isolated organs, eg. liver. This has now been achieved by using specially designed molecules (spin traps) which react with the free radicals to form more stable species (spin adducts) which can be detected using an electron spin resonance (ESR) spectrometer. With the aid of this approach, evidence has been obtained for the metabolism of carbon tetrachloride, a known hepatotoxin, to the trichloromethyl radical ($^{\bullet}CCl_3$) and a novel oxygen-containing carbon dioxide anion radical ($^{\bullet}CO_2^-$), by the intact rat liver. The appearance of these radicals correlated well with liver damage as measured by the release of the enzyme lactate dehydrogenase. In other studies the ascorbic acid (Vitamin C) radical was detected in the skin of hairless mice which had been exposed to light following treatment with the photosensitizing drug chlorpromazine. This finding implicates free radicals generated in the skin as possible toxic species responsible for skin diseases caused by the simultaneous exposure to light and chemical agents. In order to facilitate the rapid identification of spin adducts, the first database for spin trapping was designed and implemented on an IBM PC/AT. The system provides easy access to literature on spin traps, including applications to free radicals in toxicology.

Mass Spectrometry:

Mass spectrometry is a technique which is able to determine the molecular weight structures of chemical substances. It is also capable of determining the presence of very low levels of substances in complex mixtures coming from biological origins or from environmental samples. The mass spectrometry program is actively engaged in developing and applying these capabilities to problems related to the interaction of environmental toxins and biochemicals within the body. A recent project has involved the analysis of the blood of Taiwanese children whose mothers had been exposed to polychlorinated dibenzofurans (PCDF) during pregnancy. This project is important since it represents the first time that a study can be made of the fate of these compounds in human subjects. The results from this study will provide valuable information on persistence of PCDF in human tissues. PCDF are representative of a large group of chemicals, including polychlorinated biphenyls (PCB), which are a worldwide pollution problem.

Instrumental development work is currently focussed on interfacing open tubular liquid chromatography (OTLC) with mass spectrometry. Our most recent advance is the design of a coaxial interface for OTLC with continuous flow FAB. Detection levels of 20 femtomole of peptide have been achieved. Thus, this technique has potential for the real time analysis of blood levels of peptides.

Nuclear Magnetic Resonance:

Nuclear magnetic resonance (NMR) spectroscopy is a powerful analytical chemical tool which more recently has been used to study metabolic changes in cells, isolated organs and intact animals. During the past year *in vivo* NMR has been employed to delineate the hepatic metabolic pathways of two general anesthetics, halothane and methoxyflurane, which occasionally produce serious toxic side effects. In addition, the effect of the chemical additive sodium benzoate on liver metabolism has been examined. Two approaches for monitoring ions in living cells have also been developed. The first involves direct observation of intracellular cesium by NMR studies of the 100% abundant cesium-133 stable isotope. The second approach employs NMR sensitive indicators for measuring the intracellular concentrations of ions. During the past year indicators for calcium and magnesium have been developed and used to study pertubations of the levels of these ions in liver and heart cells.

In addition to these spectroscopic studies, the magnetic resonance group has recently developed capabilities for obtaining magnetic resonance images of anesthetized, experimental animals. These images provide insight into the location and physical state of all of the molecules in the cell which contain the element hydrogen. Such images are therefore dominated by the water and fat content of biological tissues. Studies to detect certain classes of highly reactive metabolic intermediates, free radical species, based on the perturbations produced on the physical properties of cell water, are currently in progress. These studies should provide new insight into the possible distribution and toxicity of these species in biological tissues.

Prostaglandin Biochemistry:

Many chemicals, which are themselves non-toxic, are chemically changed (metabolized) by enzymes in the body to more reactive forms which are highly toxic. One such important enzyme is prostaglandin H synthase (PHS) which is found in high concentrations in the bladder. Recent studies have shown that PHS metabolizes the bladder carcinogen 2-aminofluorene to a highly chemically reactive species which may be responsible for the toxicity of this compound. Other work has demonstrated that aromatic amines found in cooked food are also metabolized by PHS to free radicals. In the absence of foreign chemicals PHS converts arachidonic acid, an essential fatty acid, to prostaglandins and leukotrienes. These oxidized lipids have potent biological activities. An important recent discovery is that prostaglandins control chloride secretion. This finding may be important in uncovering the defect involved in cystic fibrosis, a hereditary disease of infants, children and young adults which is characterized by the excretion of high levels of chloride ions in the sweat.

Metabolism:

A tumor promoter does not cause cancer, but assists tumors initiated spontaneously or by a carcinogenic agent to survive and grow. An antipromoter inhibits the effects of a promoter. Some chemicals may act as promoters in some species of animals, but act as a antipromoters in other species. Prolonged activation of a protein kinase in cell membranes is thought to be part of the mechanism of action of some promoters, while antipromoters tend to prevent this activation. Our studies suggest that some compounds neither stimulate nor inhibit, but rather change the substrate specificity of this kinase in different ways in different animal species. Whether or not this species-dependent change determines the promoter/antipromoter status of the compound remains to be determined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 10004-09 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

NMR Studies of the Mechanisms of Cell Injury

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert E. London	Research Physicist	LMB	NIEHS
OTHER:	Louis Levy	Research Chemist	LMB	NIEHS
	Elizabeth Murphy	Senior Staff Fellow	LMB	NIEHS
	Michael Perlman	Senior Staff Fellow	LMB	NIEHS
	Donald G. Davis	Expert	LMB	NIEHS

COOPERATING UNITS *(if any)*

Professor Charles Steenbergen, Department of Pathology, Duke University, Durham, NC.

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Nuclear Magnetic Resonance Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	1.6	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In vivo and in situ NMR studies are carried out on systems ranging from cell suspensions to perfused organs, to intact, anesthetized experimental animals in order to determine the mechanisms by which environmental chemicals and other types of stress irreversibly injure cells. Physiological, biochemical, and magnetic resonance measurements are carried out in parallel when possible, both to validate the techniques used, and more importantly, to correlate various metabolic changes in order to determine which factors may play a causative role. It has recently been suggested that perturbations in the level of cytosolic calcium ions may be of central importance in the production of irreversible cell injury arising from a variety of agents as a consequence of the activation of various catabolic, calcium dependent enzymes such as calpain and phospholipases. The level of cytosolic calcium ions in various preparations, particularly perfused rat hearts, has been determined based on the use of fluorine-19 NMR measurements of hearts loaded with fluorinated calcium chelator. Using this approach, the diastolic calcium level was determined to be 150 mM. Cytosolic calcium rose to ~2.5 uM after 15 minutes of ischemia, a time period shorter than required to irreversibly injure the heart, as determined by the leakage of cellular enzymes. Manipulations such as the introduction of calcium channel blockers, cardioplegic arrest, and low temperature, which delay the onset of irreversible injury, all correspondingly delay the rise of cytosolic calcium.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 30003-17 LMB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of Biochemical Methodology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Phillip W. Albro	Research Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Biophysics

SECTION
Metabolism

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.4	1.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Current objectives include methods to characterize the products of the reaction of nitroxide spin traps with enzymatically generated free radicals, methods to assay for protein phosphorylation without the requirement for ^{32}P, improved assays for phospholipase C, and improved methods for the study of lipid peroxidation. An analytical high pressure liquid chromatographic method for the separation of POBN-linolate radical adducts has been developed. The fractions are characterized by their UV and FT-IR spectra, with attempts to obtain mass spectra in progress. A convenient assay for phospholipase C has been developed that permits the comparative use of a variety of substrates with low milliunit sensitivity. We are currently working on an approach to the assay of protein kinase C that will involve the use of pre-labeled protein substrates. A recommended approach to the use of scopoletin for measurement of trace levels of hydrogen peroxide gives much higher reproducibility than earlier methods.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50046-10 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Chemically Induced Photosensitivity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Anson S.W. Li	Staff Specialist	CSC	NIEHS
	Colin F. Chignell	Chief, LMB	LMB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.5	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Light is known to interact with endogenous or exogenous chemical agents in the skin or eyes, to produce photosensitization (phototoxicity or photoallergy). The objective of this study is to determine whether light-induced free radicals play a role in photosensitization. Electron spin resonance studies, in conjunction with spin trapping techniques, have shown that most halogenated aromatic photosensitizers, eg. amiodarone, bithionol, fentichlor, chlorpromazine, undergo dehalogenation upon UV irradiation to yield the corresponding aryl radicals and halogen atoms. These aryl radicals were capable of abstracting hydrogen atoms from suitable donors, suggesting that in vivo they could initiate peroxidation by reacting with unsaturated lipids. UV-irradiation of bithionol, fentichlor and related 4-chlorophenols also yielded the corresponding semiquinone radicals which may interact with the mitochondrial electron transport chain. Semiquinone radicals also disproportionate to give the parent quinones which are known to react covalently with nucleophiles (NH_2, SH). Irradiation of hairless mouse (HR/J) skin containing bithionol resulted in the generation of free radical intermediates, which may be involved in the in vivo photo-sensitization reaction to this compound. Other photosensitizers which also generate free radicals upon UV-irradiation include anthracyclines, tetracyclines, anthralin, and the salicylanilides. In order to facilitate the full use of available spin-trapping information in the literature, a database has been designed and implemented on an IBM PC/AT. The database presently contains approximately 600 references spanning a period of two decades (1967-1987).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50080-06 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Environmental Health Applications of Mass Spectrometry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
OTHER:	Carol E. Parker	Chemist	LMB	NIEHS
	Leesa Deterding	Chemist	LMB	NIEHS
	Steven McGown	Chemist	LMB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Mass Spectrometry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.15	.5	.65

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

One of the components of the mass spectrometry workgroup mission is to provide other groups within LMB and NIEHS access to mass spectrometric analyses on a service basis. The workgroup provides the following services on an ongoing basis: 1) low and high resolution electron impact (EI) mass spectra; 2) low and high resolution chemical ionization (CI) mass spectra; 3) negative ion chemical ionization (NICI) mass spectra; 4) gas chromatography/mass spectrometry (GC/MS) in conjunction with EI, CI and NICI MS; 5) thermospray (TSP) liquid-chromatography/mass spectrometry (LC/MS) in conjunction with CI and NICI MS; 6) fast atom bombardment (FAB) under both positive and negative ion conditions; 7) continuous flow FAB/MS and FAB/MS/MS under both positive and negative ion conditions; and 8) tandem MS in combination with positive and negative ion FAB, EI and CI MS.

During the past year approximately 691 analyses have been performed on a service basis (not including collaborative work).

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50082-05 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on Tumor Promoters and Antipromoters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Phillip W. Albro	Research Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Metabolism

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.6	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Current objectives include assessment of the effects of hepatic tumor promoters and antipromoters on components of the plasma membrane. Current emphasis is on effects of the compounds on protein kinase C and inositide-specific phospholipase C. Previous studies had indicated that di-(2-ethylhexyl) phthalate (DEHP), which is reportedly an antipromotor for rat liver tumors, inhibits protein kinase C *in vitro*. In extensions of these studies we have found that DEHP non-competitively prevents the activation of protein kinase C by calcium and phosphatidyl serine. This interference results from the binding of DEHP to the regulatory subunit of the kinase. The phosphorylation of proteins (such as protamine) that is not under the control of the calcium-responsive subunit is not inhibited by DEHP. The net result is a change in the substrate specificity of the enzyme. DEHP also will displace the strongly tumor-promoting phorbol diesters from protein kinase C (which serves as a phorbol ester receptor).

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50087-02 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Singlet Oxygen-Dependent Photosensitivity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert D. Hall	Staff Fellow	LMB	NIEHS
	Reza Dabestani	IRTA	LMB	NIEHS
	Colin F. Chignell	Chief, LMB	LMB	NIEHS
OTHER:	Garry R. Buettner	Expert	LMB	NIEHS

COOPERATING UNITS *(if any)*

Drs. J. B. Feix and B. Kalyanaraman, Medical College of Wisconsin, Milwaukee, WI; Dr. Ann G. Motten, Duke University, Durham, NC.

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Photosensitization can result when light interacts with endogenous or exogenous chemical agents in the skin and other tissues. This process can produce undesirable clinical consequences, as in phototoxicity and photoallergy; or it can have beneficial effects, as in tumor photodynamic therapy (PDT) and coal-tar or psoralen (PUVA) therapy against psoriasis. Photosensitization results from the light-induced production of free radicals and/or singlet oxygen ($^1O_2$), the lowest electronic excited state of molecular oxygen. Because the latter species may be important in both phototoxic reactions and PDT, we have developed state-of-the-art instrumentation capable of detecting the characteristic phosphorescence of $^1O_2$ at 1268 nm. We are also establishing a flash photolysis facility which permits us to monitor triplet-excited state activity of potential photosensitizers. This has permitted us to delineate the photophysics of $^1O_2$ production in solution by a number of chemicals with photosensitizing activities in vivo, including phenothiazine derivatives (e.g., promazine [PZ] and chlorpromazine [CPZ]), benzoxazole analogs (e.g., benoxaprofen [BP]), tetracyclines, anthrapyrazoles, anthracene, anthralin, anthraquinones, and hematoporphyrin derivative. We have shown, for example, that, when they are excited with light, PZ and CPZ sensitize significant $^1O_2$ production in organic solvents (the quantum yield, ϕ varies between 0.1 and 0.4 depending on the solvent) but they do not sensitize detectable amounts of $^1O_2$ in water ($\phi < 0.01$). Another drug, BP, also sensitizes very little $^1O_2$ production in water ($\phi < 0.01$); however, its decarboxylated photoproduct is lipid soluble and efficiently produces $^1O_2$ in organic solvents. We have also demonstrated that upon irradiation Merocyanine 540, currently used to destroy tumor cells in bone marrow explants, produces $^1O_2$ in liposomes and that anthracene, eosin Y and hematoporphyrin derivative produce $^1O_2$ in erythrocyte ghosts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50088-02 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Relationship of Free Radicals to Halocarbon-Induced Toxicity in the Liver

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and insitute affiliation)*

PI:	Ronald P. Mason	Research Chemist	LMB	NIEHS
OTHER:	Kathy T. Knecht	Biologist	LMB	NIEHS
	Henry D. Connor	Research Chemist	LMB	NIEHS
	David Duling	Programmer/Analyst	CSC	NIEHS

COOPERATING UNITS *(if any)*

Dr. Ronald G. Thurman, Department of Pharmacology, UNC, Chapel Hill, NC

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

CCl_4 has been shown previously to be metabolized to the trichloromethyl radical ($\cdot CCl_3$) and to a novel oxygen-containing carbon dioxide anion radical ($\cdot CO_2^-$) in the perfused rat liver. These free radicals were detected by electron spin resonance using the spin-trapping technique. The $\cdot CO_2^-$ radical adduct also was observed in urine following the intragastric administration of CCl_4 or $CBrCl_3$ and spin trap. Detection of the $\cdot CO_2^-$ adduct in the effluent perfusate was decreased 3-4 fold by DIDS (0.2 mM), an inhibitor of the plasma membrane anion transport system. The rate of formation of $\cdot CO_2^-$ radical adduct was decreased 2-3 fold following inhibition of cytochrome P-450-dependent mono-oxygenases by metyrapone (0.5 mM) and was increased about two-fold by induction of cytochrome P-450 by phenobarbital pretreatment. Toxicity of halocarbons in the perfused liver was assessed by measuring the release of lactate dehydrogenase (LDH) into the effluent perfusate in livers from phenobarbital-treated rats under conditions identical to those employed to detect radical adducts (i.e., during the infusion of CCl_4 or $CBrCl_3$ into livers perfused with either nitrogen- or oxygen-saturated perfusate). Metabolism of halocarbons to $\cdot CO_2^-$ radical adduct was 6-8 fold faster during perfusion with nitrogen-saturated rather than with oxygen-saturated perfusate. Concomitantly, liver damage detected from LDH release occurred much sooner during halocarbon infusion in the presence of nitrogen-saturated perfusate. A good correlation (r= -0.80) between the rate of formation of PBN/$\cdot CO_2^-$ and the time to onset of LDH release following halocarbon infusion was observed. Therefore, it is concluded that PBN/$\cdot CO_2^-$ is a useful marker for oxygen-containing free radical intermediates which may be causally related to halocarbon-induced hepatotoxicity. Recently, the $\cdot CCl_3$ and $\cdot CO_2^-$ radical adducts also have been detected in the bile from anesthetized rats.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50089-02 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Reaction of Free Radical Metabolites with DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ronald P. Mason	Research Chemist	LMB	NIEHS
OTHER:	William D. Flitter	Visiting Fellow	LMB	NIEHS
	David Duling	Programmer/Analyst	CSC	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The interaction of free radical metabolites with DNA has been a major area of interest and speculation, but previous electron spin resonance (ESR) investigations of this area have been very limited. The reaction of the hydroxyl radical, generated by a Fenton system, with pyrimidine deoxyribonucleotides was investigated using the ESR technique of spin trapping. The spin trap *t*-nitrosobutane was employed to trap secondary radicals formed by the reaction of the hydroxyl radical with these nucleotides. The results presented here show the hydroxyl radical attack on thymidine, 2-deoxycytidine 5-monophosphate and 2-deoxyuridine 5-monophosphate produced nucleotide-derived free radicals. The results indicate that $\cdot$OH radical attack occurs predominantly at the carbon-carbon double bond of the pyrimidine base. The ESR studies showed a good correlation with previous work produced by authors who used x- or γ-ray irradiation to generate the hydroxyl radical. A thiobarbituric acid assay was also used to monitor the damage produced to the nucleotides by the Fenton system. These results showed qualitative agreement with the spin trapping studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50090-02 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Porphyrin Ion Radical Metabolites and Their Reactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ronald P. Mason	Research Chemist	LMB	NIEHS
OTHER:	Herbert Sipes	IPA	LMB	NIEHS
	David Duling	Programmer/Analyst	CSC	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Uroporphyrin I, which accumulates in body tissues of congenital erythropoietic porphyria patients, can undergo an enzymatic one-electron reduction to the porphyrin anion radical when a suitable reducing cofactor is present. We have demonstrated that anaerobic microsomal incubations containing NADPH and uroporphyrin I give an electron spin resonance spectrum of a porphyrin anion free radical. Inhibitor studies indicate that NADPH-cytochrome P-450 reductase is the electron donor. This radical undergoes a second-order decay due to nonenzymatic disproportionation of the radical. Aerobic microsomal incubations were also investigated for the reduction of oxygen to superoxide by monitoring oxygen consumption and the spin-trapping of superoxide. These experients demonstrated that electron transfer from the porphyrin radical to molecular oxygen does occur, but due to the slow formation of the radical anion, no oxgyen consumption above the basal level could be detected in the microsomal incubations. The photoreduction of uroporphyrin I in aerobic and anaerobic incubations was also investigated. Similar results have been obtained with photofrin II, a photo-activated antitumor agent. The oxidation of a variety of porphyrins to cation free radicals by peroxidases also has been investigated. Since the enzyme intermediate of horseradish peroxidase, compound I, is itself a porphyrin IX cation radical, this work will have implications for electron transfer as well as porphyrin metabolism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50091-02 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Phenyl Radical Formation by Oxyhemoglobin from Phenylhydrazine In Vivo

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ronald P. Mason	Research Chemist	LMB	NIEHS
OTHER:	Kirk R. Maples	Staff Fellow	LMB	NIEHS
	Sandra Jordan	Biologist	LMB	NIEHS
	David Duling	Programmer/Analyst	CSC	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The reaction of oxyhemoglobin with phenylhydrazine has received considerable attention for many decades. The basis for this interest stems from the ability of phenylhydrazine and hydrazine-based drugs to induce hemolytic anemia. Considerable evidence obtained from in vitro electron spin resonance (ESR) experiments implicates free radicals in the events leading to red blood cell hemolysis. However, until this report, no corroborating ESR evidence for in vivo free radical formation has been presented. We have successfully employed ESR to detect the formation of a radical adduct in the blood of rats which received an intragastric dose of phenylhydrazine followed by an intraperitoneal injection of the spin trap 5,5-dimethyl-1-pyrroline N-oxide (DMPO). The results of a series of experiments with sulfhydryl reagents and C-13-labelled phenylhydrazine led us to assign this DMPO radical adduct to the trapping of a hemoglobin-derived thiyl free radical. In addition to phenylhydrazine the hydrazine-based drugs isoniazid, iproniazid, phenelazine, and hydralazine were examined. Of the four drugs, only phenelzine and iproniazid were able to induce the formation of the DMPO/hemoglobin thiyl free radical adduct in vivo, whereas only phenelzine and hydralazine were able to form this adduct in vitro. We were able to decrease the in vivo iproniazid-induced adduct formation by pretreating the rats with bis-para-nitrophenylphosphate, an arylamidase inhibitor. Our results support the idea that iproniazid is hydrolyzed in the liver to a more reactive metabolite, most likely isopropylhydrazine, which is subsequently released into the blood stream.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50092-02 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Mechanisms of Reduction of Nitroheterocyclic Drugs

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ramakrishna Rao	Visiting Associate	LMB	NIEHS
OTHER:	Ronald P. Mason	Research Chemist	LMB	NIEHS
	Sandra Jordan	Biologist	LMB	NIEHS
	David Duling	Programer	CSL	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The first step in the metabolic conversion of a nitroheterocyclic drug is the reduction of the nitro group to give the corresponding nitro radical anion. Several reductases and low-molecular weight bio-molecules are known to reduce nitro compounds. We have shown in the present work that the nitroheterocyclic drugs can be reduced by intact rat hepatocytes, and the nitro radicals were formed inside the cell. By using line-broadening paramagnetic reagents, we have shown that the nitro radicals formed inside the cell "freely" cross the plasma membrane, and the radical detected by ESR is mainly due to the radical present outside the cell. This approach of using paramagnetic reagents to distinguish the nitro radical anion present inside the cell from those outside the cell can also be used to study radical cations. Hence, we studied the formation of bipyridylium radical cations by hepatocytes. Our results for these radicals are the same as that for nitro radical; namely, bipyridylium radicals are formed inside the cell, but cross the plasma membrane. The radicals detected by ESR are mostly present outside the cell. The radicals present inside the cell were detected when the ESR signal due to the radicals present outside was completely broadened.

Monoglutathionyl 1,4-benzosemiquinone radical formed by rat hepatocytes in the presence of 1,4-benzoquinone is shown to be formed inside the cell, but again the radical detected by ESR is outside the cell. Glutathionyl conjugates are known to be exported by hepatocytes.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50093-02 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

NMR Studies of the Metabolism of Leishmania Braziliensis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert E. London	Research Physicist	LMB	NIEHS
OTHER:	Donald G. Davis	Expert	LMB	NIEHS

COOPERATING UNITS *(if any)*

Professor J.J. Blum, Division of Phyisology, Department of Cell Biology, Duke University Medical Center, Durham, NC. 27710

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Nuclear Magnetic Resonance Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.6	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Although Leishmania species are estimated to infect about 100 million people worldwide, little is known about the biochemistry of these microorganisms. We have continued to carry out metabolic studies based on the use of metabolic precursors labeled with the stable, NMR sensitive carbon-13 isotope, combined with NMR detection of excreted and extracted metabolites. During the past year, efforts were made to further define the anaerobic metabolism under a variety of conditions. In a N_2 atmosphere, glycerol is the major excreted metabolite, with a large pool of glycerol-3-phosphate detectable in cell extracts. In an atmosphere of N_2 plus CO_2, succinate is the major product, and glucose consumption occurs at a rate comparable to that observed in O_2 or O_2 + CO_2 atmospheres. The consumption of glucose under anaerobic conditions in the presence of CO_2 is driven by the formation of malate and NAD^+ derived from the reduction of oxalacetate, which in turn is formed from phosphenolpyruvate plus the CO_2. The significance of this pathway is further indicated by studies utilizing ^{13}C labeled CO_2, in which the label is observed at positions 1 and 4 of succinate. Initial observations on the production of D-lactate have been extended to a demonstration of its formation via methylglyoxal synthase and glyoxalases I and II. D-lactate dehydrogenase does not appear to be present. Initial ^{31}P NMR studies of cell extracts have established the presence of a large pool of polyphosphates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50094-02 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

NMR Studies of Dihydrofolate Reductase

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert E. London	Research Physicist	LMB	NIEHS
OTHER:	Barry Selinsky	Staff Fellow	LMB	NIEHS
	Michael S. Perlman	Senior Staff Fellow	LMB	NIEHS

COOPERATING UNITS *(if any)*

Dr. Raymond L. Blakley, Chairman, Division of Biochemical and Clincial Pharmacology, St. Jude Children's Research Hospital, Memphis, TN.

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Nuclear Magnetic Resonance Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.7	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Interest in the enzyme dihydrofolate reductase (DHFR) derives in part from its clincial relevance as a target enzyme of anti-folate drug therapy. The reductase is inhibited by methotrexate, used in the treatment of various types of cancer, and the bacterial enzyme is strongly and specifically inhibited by trimethoprim, which is used in combination with sulfa drugs for treating a variety of infectious diseases. Studies carried out during the past year have been aimed at elucidating the catalytic mechanism of this enzyme by carrying out NMR analyses on the binary complex formed by the enzyme and isotopically labeled dihydrofolate. These studies have been motivated by proposals that protonation of the bound dihydrofolate facilitates the subsequent hydride transfer reaction from NADPH to yield the reduced tetrahydrofolate product plus NADP+. Based on speculation that this protonation is occurring at the N-5 position of the pterin ring system, derivatives containing nitrogen-15 at the N-5 position and carbon-13 at the C-6 position have been synthesised. As a consequence of the limited solubility of dihydrofolate, labeled analogs have also been prepared, including the pentaglutamyl derivative of dihydrofolate, and dihydrobiopterin. A series of pH dependent studies carried out on the complexes formed between the labeled dihydrofolate and analogs and the beef liver dihydrofolate reductase suggest that the general model based on N-5 protonation may be incorrect. Further studies of this important question are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50095-02 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

In Vivo F-19 NMR Studies of the Metabolism of Fluorinated Anesthetics

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Robert E. London	Research Physicist	LMB	NIEHS
OTHER:	Barry S. Selinsky	Staff Fellow	LMB	NIEHS
	Michael Perlman	Senior Staff Fellow	LMB	NIEHS
	Scott A. Gabel	Biologist	LMB	NIEHS
	Donald G. Davis	Expert	LMB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Nuclear Magnetic Resonance Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.7	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The liver is the principal organ involved in the detoxification of a broad range of xenobiotics. During the past decade, it has become possible to monitor the metabolism of organs non-invasively in anesthetized animals and humans using surface coil NMR probes which can be placed directly over the tissue of interest. Using this technique, the metabolic transformations of xenobiotics can, in favorable cases, be directly observed, and perturbations of cellular energy metabolism and redox chemistry can also be studied. Using this technique, we have carried out studies of metabolism of several fluorinated inhalation anesthetics in order to obtain a better understanding of the basis for the toxic side effects which accompany the use of many of these compounds. These studies have provided the first unequivocal identification of methoxydifluoroacetate as a metabolite of the anesthetic methoxyflurane. Additionally, we have adapted this technique to the study of transmethylation reactions based on direct observations by deuterium NMR of [methyl-$^2H_3$]methionine. It was found that excess methionine is metabolized largely via the initial transfer of the methyl group to glycine to yield N-methylglycine (sarcosine), which is subsequently processed in the mitochondrion. The activity of this glycine/sarcosine shuttle is subject to interference by a number of chemicals including the methionine analog ethionine, as well as sodium benzoate. Studies with deuterated D-methionine indicate that this analog is metabolized via initial oxidative deamination by D-amino acid oxidase, then transaminated to yield L-methionine which is then metabolized via glycine N-methyltransferase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50096-02 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Changes in Tissue Non-Cyclic Phosphodiesters Produced by Toxins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	C. Tyler Burt	Expert	LMB	NIEHS
OTHER:	Robert E. London	Research Physicist	LMB	NIEHS
	Phillip W. Albro	Research Chemist	LMB	NIEHS

COOPERATING UNITS (if any)

Dr. Charles Hill, Dept. Poultry Sci., NCSU, Dr. Jay Levine, N.C. Vet. School, Raleigh, NC; Drs. M. Abou-Donia and Clark Carrington, Dept. Pharmacology, Duke Univ., Durham, NC; Dr. T. Ng, Dept. of Radiology, Cleveland Clinic, Cleveland, OH

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Nuclear Magnetic Resonance Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.1	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In vivo 31-P nuclear magnetic resonance (NMR) techniques have been used to evaluate the effects of several toxic substances on tissue metabolism, with particular emphasis on the potential significance of changes in the levels of non-cyclic phosphodiesters. In mammalian systems, the phosphodiesters glycerophosphoryl choline (GPC) and glycerophosphoryl ethanolamine (GPE) are readily observed in many tissues and hence are present at near millimolar levels. Although most generally postulated to be lipid catabolites, it is difficult to reconcile this lack of function with the high tissue levels observed. Further, the presence of analogous phosphodiesters such as serine ethanolamine phosphodiester (SEP) which is found in the chicken, and threonine ethanolamine phosphodiester (TEP) which we have found to be the major phosphorus-containing metabolite in fish lens, also suggests functions beyond that of lipid breakdown product. In the most recent studies it appears that TEP might be present in some mammalian tumors. To investigate this, authentic TEP has been isolated from fish lens and will be compared to the resonance in tumor extracts. Since fish tissue contains the novel lipid phosphatidyl threonine, studies arrived at characterizing the corresponding pH and ^{31}P resonances are in progress. Finally to expand the range of toxins investigated, the neurotoxin triphenylphosphite, has been studied. In particular, its influence on tissue at the site of injection has been characterized by surface coil analysis. It was possible not only to examine the intercellular phosphates but also to follow the course of absorption of the toxin.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 50097-02 LMB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development and Application of an OTLC-MS Interface

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
OTHER:	Jos de Wit	Chemist	LMB	NIEHS
	Arthur Mosley	Chemist	LMB	NIEHS
	Carol E. Parker	Chemist	LMB	NIEHS
	Bernard Escoffier	Visiting Fellow	LMB	NIEHS
	Leesa Deterding	Chemist	LMB	NIEHS
	Steven McGown	Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*
Professor James Jorgenson, Department of Chemistry, UNC, Chapel Hill, NC.

LAB/BRANCH
Laboratory of Molecular Biophysics

SECTION
Mass Spectrometry

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.58	.73	1.85

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An interface has been developed for the direct coupling of Open Tubular Liquid Chromatography (OTLC) and Mass Spectrometry. The interface has similarities to both direct liquid introduction (DLI) and thermospray (TSP) interface designs. The interface is introduced into the vacuum system via the solid probe inlet; thus, it does not compromise the use of other inlet systems, such as GC, DLI, or direct probe.

The capabilities of this interface have been extended to magnetic sector instruments. The presence of a high potential electric field has aided in the ionization of compounds, such as fatty acids, amino acids and bases such as adenine which could not be analyzed on a quadrupole MS.

The concept has been extended to continuous flow FAB/MS where the OTLC column is encased in a sheath column. The sheath column delivers the matrix to the probe tip while the inner column delivers the analyte. Detection levels of low programs for a tripeptide have been achieved.

We are currently modifying this CF/FAB design for use in capillary zone electrophoresis (CZE) MS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50098-02 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of FAB/MS and FAB/MS-MS for Environmental Health Sciences

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
OTHER:	Sunita Verma	Visiting Fellow	LMB	NIEHS
	Leesa Deterding	Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

Professor Carol Djerassi, Stanford University, CA, Professor Arno Spatola, University of Louisville, KY.

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Mass Spectrometry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	.75	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Fast atom bombardment (FAB) has become one of the most widely utilized techniques for obtaining mass spectra of thermally labile and/or very polar substances. In conjunction with tandem MS (MS-MS), the fragmentation information so vital for structure elucidation can be obtained from ions normally not observed to fragment and from mixtures where the parent ion-daughter ion relationships are not known.

This project involves instrumental developments in MS-MS and application of FAB/MS and FAB/MS-MS for structure elucidation. In the area of instrumental developments we are currently investigating the effect of energy loss on the MS-MS data obtained by a linked scan of MS-II and for how these effects can best be compensated.

Applications of FAB/MS and FAB/MS/MS for structure elucidation include: the structure elucidation of phospholipids containing unusual fatty acid and headgroups; peptides in which an amide linkage has been modified; and glutathione, cysteine and N-acetylcysteine conjugates of xenobiotics, both synthetic and conjugates isolated from challenged animals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50099-02 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Application of Thermospray LC-MS to Structure Elucidation of Biomolecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
	Carol E. Parker	Chemist	LMB	NIEHS
	Bernard Escoffier	Visiting Fellow	LMB	NIEHS
	Sunita Verma	Visiting Fellow	LMB	NIEHS
OTHER:	Jos de Wit	Chemist	LMB	NIEHS
	L.T. Burka	Research Chemist	DTRT/STB	NIEHS
	F. Kari	Research Chemist	DTRT/CTEB	NIEHS

COOPERATING UNITS *(if any)*

Professor Buhler, Oregon State University

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Mass Spectrometry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.58	1.48	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The majority of biochemical samples are complex mixtures. Separation and isolation of each component prior to analysis is often laborious and tedious. Therefore, considerable effort has been expended on the development and application of methodologies designed to separate and introduce each separated component of a mixture directly into a mass spectrometer. GC/MS, used for the separation and analysis of volatile and thermally stable substances is a relatively mature technique. LC/MS, which combines liquid chromatographic separation with MS analysis is still at a stage where several interfaces are in popular use and where the capabilities of the technique in its various manifestations have not been thoroughly explored.

We are currently exploring the applicability of thermospray (TSP) as an LC-MS interface for biochemical problems. Several studies are being carried out concurrently including: 1) determination of glutathione and related conjugates (cysteine, N-acetylcystene) of exogenous chemicals; 2) analysis of the highly toxic senecionine alkaloids and their microsomal metabolites; 3) analysis of the herbicide metribuzine and its plant metabolites; 4) ANF (α-naphthoflavone) metabolites; and 5) the metabolic products of H.C. Blue No. 1 and H.C. H.C. Blue No. 2 (H.C. Blue No. 1 is a carcinogen previously used in hair dye.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50100-02 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure Elucidation of Carcinogen-Nucleoside Adducts

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
OTHER:	Leesa Deterding	Chemist	LMB	NIEHS
	John Dino, Jr.	Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

Andrea Dietrich, Guest Worker, Dr. L.M. Ball, A. Bartczak, Dr. A. Gold, Dept. Env. Sci., Professor D.G. Kaufman, Dept. of Pathology, UNC, Chapel Hill, NC; Drs. S. Nesnell and S. Agarwal, USEPA, Research Triangle Park, NC;

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Mass Spectrometry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.75	.1	.65

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

One of the major research concerns at NIEHS is to understand the interactions between exogenous chemicals and biomolecules within the body. For a complete understanding of this interaction, the structures of the products of covalent interactions must be elucidated. Of special concern are the products of reactions between environmental carcinogens and DNA.

The products of the _in vitro_ reaction of benzo[a]pyrenediol-epoxide and oligonucleotides have been successfully analyzed by FAB/MS and FAB/MS/MS. Negative ion MS/MS gives sequence information identifying the bases which were modified while positive ion FAB identified the site of modification on the base.

The products of the enzymatically mediated reaction of 3-nitrofluorene and DNA has been determined to be (after nuclease digestion) N-(deoxyguanosin-8-yl)3-aminofluoranthene. We are currently analyzing the 3-methyl-chrysenediol epoxide adduct with guanylic acid.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50101-02 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Identification of Tetrachlorodibenzofuran Metabolites

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
OTHER:	Carol E. Parker	Chemist	LMB	NIEHS
	L.T. Burka	Chemist	DTRT/STB	NIEHS

COOPERATING UNITS *(if any)*

DTRT/STB

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Mass Spectrometry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is currently on.hold pending the isolations of a quantity of analytic sufficient for the analysis of the lower concentration metabolites.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50102-02 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mass Spectral Analysis of the Benzo[a]Pyrene-DNA Reaction Product

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Leesa Deterding	Chemist	LMB	NIEHS
OTHER:	John Dino, Jr.	Chemist	LMB	NIEHS
	Kenneth B. Tomer	Research Chemist	LMB	NIEHS

COOPERATING UNITS (if any)

Professor David G. Kaufman, Department of Pathology, UNC, Chapel Hill, NC

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Mass Spectrometry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.06	.05	.55

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been combined with Z01 ES 50100-02 LMB.

PHS 6040 (Rev 1/84) GPO 914-919

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50103-02 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

GC-MS Analysis of PCDF Blood Levels in Children Exposed In Vitro

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Walter Rogan	Chief, Epidemiology Branch	DRA/EB	NIEHS
OTHER:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
	Steven McGown	Chemist	LMB	NIEHS

COOPERATING UNITS (if any)

Dr. Linda Sheldon, RTI, Research Triangle Park, NC

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Mass Spectrometry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.05	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

There have been two outbreaks of human poisoning by polychlorinated biphenyls (PCBs) and their thermal breakdown products; the first, in Japan in 1968, the second in Taiwan in 1979. Because PCBs are a world wide pollution problem, these episodes have been studied carefully, since they have presented the only opportunity to observe directly the toxicity of PCBs in human beings outside the workplace. Laboratory methods for the evaluation of these outbreaks were relatively unsophisticated in 1968; there has been great progress in analytical methods since. In collaboration with Taiwanese scientists, the Epidemiology Branch, NIEHS, had the opportunity to examine over 100 children who had been in utero at the time of the 1979 poisoning or afterward. These children continued to be affected, since the chemicals cannot be excreted from the mother's body.

In a collaborative effort, the mass spectrometry workgroup will analyze approximately 120 serum samples for tetra-, penta- and hexachlorodibenzofurans at the part per trillion level. We have currently validated the extraction procedure and analysis of the samples will commence in FY 1989.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50104-02 LMB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
In Vivo NMR Studies of Cellular Magnesium

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert E. London	Research Physicist	LMB	NIEHS
OTHER:	Louis Levy	Research Chemist	LMB	NIEHS
	B. Raju	Visiting Fellow	LMB	NIEHS
	Elizabeth Murphy	Senior Staff Fellow	LMB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Biophysics

SECTION
Nuclear Magnetic Resonance Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	1.9	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It is generally recognized that cytosolic ions play a significant role in the regulation of cellular metabolism, and that disturbances in ionic homeostases resulting from exposure to enviornmental chemicals or heavy metals may be a significant factor in explaining observed toxicity. Although interest in the regulatory role of metals ions has chiefly centered around cytosolic calcium, there is an increasing appreciation of the role which cytosolic magnesium ions play in physiological and pathophysiological processes. Magnesium ions are reported to modulate many cell activities such as adenylate cyclase, calcium release from the sarcoplasmic reticulum in heart, and the plasma membrane K channel. An evaluation of the role of cytosolic magnesium as an acute or chronic regulator of cell function has been hampered by the lack of suitable methods for monitoring it. We have developed a series of intracellular indicators for magnesium which have dissociation constants in the physiological range, and dissociation constants for other cellular ions which are well above the physiological concentrations. One series of intracellular indicators contains the element fluorine and can be detected using fluorine-19 nuclear magnetic resonance. The resulting series of chelators: 4-fluoro, 5-fluoro, and 4-methyl-5-fluoro o-aminophenol-N,N,O-triacetate (APTRA) have been characterized. A second approach combines the fluorophores developed by Tsien with the APTRA structure to yield fluorescent indicators. The indicator 2-[2-(5-carboxy)oxazole]-5-hydroxy-6-aminobenzofuran-N,N,O-triacetate (FURAPTRA) has been evaluated and the dissociation constants for Ca and Mg ions were determined to be 53 μM (well above the physiological level) and 1.5 mM (near the physiological level), respectively.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50105-01 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Airway Epithelium Prostaglandins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
OTHER:	Steven McGown	Chemist	LMB	NIEHS

COOPERATING UNITS (if any)

Dr. David Henke, Dept. of Pulmonary Medicine, University of North Carolina Medical School, Chapel Hill, NC

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Mass Spectrometry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.25	.05	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aims of this project are to determine whether or not human airway epithelium tissues produce prostaglandins and determine the cell mediated turnover of exogenous arachidonic acid.

The question of prostaglandin production arises in view of differing results from cultured airway epithelium cells and from fresh cells.

The Mass Spectrometry Workgroup will undertake the derivatization and analysis of the prostaglandin fractions isolated from the cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80008-14 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biosynthesis of Prostaglandins, Hydroxy-Fatty Acids and Leukotrienes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Thomas E. Eling	Research Chemist	LMB	NIEHS
OTHER:	Wayne Glasgow	IRTA	LMB	NIEHS
	Roberta Danilowicz	Biologist	LMB	NIEHS
	Jeff Handler	IRTA	LMB	NIEHS
	Mike Luster	Research Microbiologist	TRTP	NIEHS
	Tim Bertnam	IPA	LPP	NIEHS
	Arnold Brody	Research Biologist	LPP	NIEHS
	Julie Angerman-Stewart	Biologist	LMB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Prostaglandin Biochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS: 3.9	PROFESSIONAL: 2.7	OTHER: 1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Investigations are concerned with the oxidation of arachidonic acid to prostaglandins (PG), leukotrienes and hydroxy-fatty acids and the relationship of this metabolism to the regulation or modulation of biological processes. Human airway cells were found not biosynthesize PGs but to make 15-HPETE and its metabolites. Arachidonic acid metabolism by porcine alveolar and intravascular macrophages is very different with intravascular more active than alveolar. We have also investigated the mechanism responsible for the inhibition of PHS by phenylbutazone (Pb). Pb must be oxidized by PHS peroxidase for inhibition to occur. Pb- peroxide was not a substrate for PHS peroxidase nor an inhibitor. The data indicates that Pb alkoxyl or peroxyl radicals are responsible for the inhibition. We have also investigated the role of arachidonic acid metabolism in mitogenesis. Mouse B lymphocytes oxidized arachidonic acid to 12-HPETE and 12-HETE. Inhibitors of 12-lipoxgenase inhibit lipopolysaccharide (LPS) dependent mitogenesis. The addition of cofactors for peroxidase inhibited mitogenesis without altering the total oxidation of arachidonic acid. A new arachidonic acid metabolite produced by lymphocytes has been isolated characterized as a mixture of 19/20 hydroxy-12-HETE with one or more double bond reduced. Further studies are required to determine the position and extent of reduction. We have also studied arachidonic acid metabolism and EGF induced mitogenesis in BALBc 3T3 cells. EGF induces the formation of PGE_2 (the major arachidonic acid metabolite) and mitogenesis. Indomethacin, dexamethasone, and NDGA block PGE_2 formation and mitogensis. Prostaglandins are weakly mitogenic but the addition of PGs to indomethacin inhibited EGF-stimulated mitogenesis restored DNA synthesis. Moreover, the addition of PGs greatly enhance EGF mitogenesis. These studies suggest a possible important role for arachidonic acid metabolism in regulating cell growth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80035-12 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cooxidation of Xenobiotics by the Prostaglandin Synthetase

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Thomas Eling	Research Chemist	LMB	NIEHS
Other:	Ronald Mason	Research Chemist	LMB	NIEHS
	David Thompson	Staff Fellow	LMB	NIEHS
	Gary Foureman	Staff Fellow	LMB	NIEHS
	John Curtis	Chemist	LMB	NIEHS
	Julie Angerman-Stewart	Chemist	LMB	NIEHS
	Yolanda Van der Zee	Visiting Fellow	LMB	NIEHS

COOPERATING UNITS (if any)

Michael Hughes, UNC Post-doctoral Fellow, Chapel Hill, NC

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Prostaglandin Biochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.7	2.9	1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The long range goal of this project is to study the oxidation of chemicals to toxic or carcinogenic metabolites by prostaglandin H synthase (PHS) and to demonstrate the importance of this enzyme system in chemical-induced toxicity or carcinogensis. We have shown that aromatic amine carcinogens, are metabolized to mutagens by PHS. PHS dependent oxidation occurred by a free radical mechanism and resulted in the formation of DNA adducts which can be used as in vivo markers for PHS-dependent oxidation. We have further studied the formation of amine mutagens by PHS using bacterial tester system that are rich or devoid of acetylase activity. Our data indicates that acetylase plays an important role in the formation of free radical mutagens. We have also studied the epoxidation of BP-7,8-diol by peroxyl radicals generated during bisulfite auto oxidation or catalysis by peroxidase. Significant enhancement of diol epoxidation occured and significant formation of sulfite conjugates were observed. The data may explain the co-carcinogensis between bisulfite (SO_2) and benzo(a)pyrene. We have also discovered a new mechanism for the formation of glutathione adducts that occurs in the absence of epoxide formation and conjugation by glutathione transferase. By this mechanism the model compound, styrene, reacts with a thiyl radical formed enzymatically by PHS. The resulting styrene-glutathione carbon-centered radical then reacts with molecular oxygen to form a peroxyl radical which eventually yields the conjugate. We have also examined the mechanism involved in the inhibition of peroxidase by methimazole. Our data indicates that methimazole is not a peroxidase inhibitor as reported, but inhibit these reactions by reducing free radical metabolites to parent compound. The results in the net inhibition of metabolism. Our data suggest that PHS is a versatile enzyme system that can catalyze a variety of reactions and appears to be important in conversion of chemicals to carcinogenic metabolites in extra hepatic tissue.

LABORATORY OF MOLECULAR CARCINOGENESIS

LABORATORY OF MOLECULAR CARCINOGENESIS
Summary Statement

The overall goal of this new Laboratory established in December 1987 is to understand the genes involved in carcinogenesis, including proto-oncogenes and tumor suppressor genes, and to elucidate the mechanisms by which environmental substances affect these genes. The major research efforts of the Laboratory include: (1) cloning and characterizing tumor suppressor genes; (2) understanding the interactions between oncogenes and tumor suppressor genes; (3) cloning transforming genes from human and rodent mesotheliomas; and (4) understanding the factors involved in the susceptibility of $B6C3F_1$ mice to hepatocarcinogenesis.

A number of major accomplishments have been made recently by LMC. The major focus of the Laboratory has been on tumor suppressor genes, and we have shown that loss or inactivation of tumor suppressor genes is an essential step in the multistep neoplastic transformation of Syrian hamster embryo (SHE) cells. Nontumorigenic variants have been isolated which have lost (sup^-) or retained (sup^+) the ability to suppress tumorigenicity of tumor cells in cell hybrids. Fusions of sup^+ or sup^- variants with different tumor cells show different patterns of suppression indicating that a family of tumor suppressor genes exists in these fibroblast cells.

Currently, several strategies to clone tumor suppressor genes are in progress. A novel gene transfer assay has been developed using a cDNA library prepared from normal human fibroblasts in a mammalian expression vector (kindly provided by Dr. H. Okayama), coupled with a highly efficient transfection procedure and a negative selection method. The library was transfected into tumorigenic cells and after G418 selection, transfectants which grow in media containing 1% serum and BUdR are killed by near-UV light treatment, while growth suppressed transfectants survive and are isolated. DNAs from these surviving revertants are subjected to an additional round of transfection and negative selection. Appropriate secondary transfectants will be utilized to recover the tumor suppressor cDNA by one of several cloning techniques. Another strategy involves cDNA libraries of sup^+ hamster cells which have been screened with RNA from sup^+ or sup^- cells. Currently, 31 differentially expressed clones have been isolated and are being characterized and three show excellent correlation with the sup^+ phenotype. Further, subtractive hybridization techniques are being employed to construct a eukaryotic expression vector library enriched for potential tumor suppressor cDNAs. Ultimately, this enriched library will be analyzed by transfection as described above. In related studies, [^{35}S] methionine-labeled proteins in total lysates of cells at various stages of transformation have been examined in collaboration with Dr. M. Lambert (Cold Spring Harbor) for alterations in gene expression by two-dimensional protein gel analysis. These reveal only limited differences between sup^+ and sup^- cells among over 1000 proteins analyzed, and a dramatic reduction of several tropomyosin isoforms was correlated with the sup^- phenotype.

A cellular phenotype associated with the loss of tumor suppressor gene function has also been found. Sup^- cells suspended in agar respond reversibly to transforming and normal growth factors by forming anchorage-independent colonies whereas sup^+ cells fail to grow. In addition to the tumor suppressor genes described above which are expressed in some immortal cell lines, tumorigenicity also may be limited by cellular senescence. Attempts to map the senescence phenotype to a particular human chromosome are in progress. A single human chromosome (#1) is preferentially lost in hybrids between normal human fibroblasts and immortal hamster cells when the hybrids escape senescence. Microcell experiments to transfer this chromosome into immortal cells and induce senescence are in progress. Similar experiments to define tumor suppressor genes for human mesotheliomas and small cell lung cancers also are planned.

Proto-oncogene activation during carcinogen-induced neoplastic transformation of SHE cells has also been studied. DNAs from ~50% of the tumor cell lines examined were positive in the NIH 3T3 focus assay and the transforming gene was identified as H-ras. These activated H-ras genes were observed in tumor cells but not in the immortal, nontumorigenic cells, suggesting that ras activation is a late, post-immortalization step in the neoplastic progression of these cells. Several chemically transformed hamster tumor cell lines also display a 4- to 11-fold increase in the specific activity of pp60^{c-src} kinase compared with preneoplastic and normal SHE cells. When chicken pp60^{c-src} was transfected into one tumor cell line, a 3-fold activation of the exogenous tyrosine kinase activity was observed compared with the chicken pp60^{c-src} expressed in a preneoplastic cell line, suggesting that the activation mechanism involves a trans-acting factor.

The abilities of DNAs from human mesotheliomas to neoplastically transform NIH 3T3 cells were examined. Cultures transfected with mesothelioma DNAs formed tumors in nude mice under conditions where control cultures were negative. Southern analyses of these nude mouse tumors for human sequences homologous to the H-ras, K-ras or N-ras oncogenes were negative, indicating that the activated transforming genes are not members of the ras gene family, but the primary and secondary transfectants did contain human sequences detectable by hybridization to a human Alu repetitive DNA probe. Further experiments to characterize and clone these genes by cosmid rescue are in progress.

Current research of the Chemical Carcinogenesis Group headed by Dr. Roger Wiseman is focused on understanding the susceptibility of the B6C3F$_1$ mouse to hepatocarcinogenesis. More than 140 B6C3F$_1$ hepatomas, which arose spontaneously or were induced by a single injection of one of 12 carcinogens during the preweanling period, have been examined for proto-oncogene activation. Approximately 90% of these hepatomas were shown to contain activated alleles of the H-ras proto-oncogene which could induce morphological transformation of NIH 3T3 cells; K-ras proto-oncogenes were activated in another 5% of these tumors. The H-ras activating mutations were characterized at the DNA sequence level by selective oligonucleotide hybridization and digestion with restriction enzymes diagnostic for specific base substitutions within codon 61. The observation of carcinogen-specific mutation patterns indicated that these lesions resulted from the direct interaction of electrophilic ultimate carcinogens with this gene in vivo. Since these tumors were induced by a single carcinogen treatment early in life, these mutations may represent an initiating event of hepatocarcinogenesis in this model system. As a test of this hypothesis, transgenic C57BL/6N mice will be constructed to contain an activated H-ras gene from an N-nitrosomethylurea-induced rat mammary carcinoma. Transgenic lines will be developed in the C57BL/6N background and crossed with normal C3H/HeN mice. Hepatoma incidence (as well as other tumors) in the parental lines and hybrid offspring will be compared to ascertain whether the C3H/HeN genetic background is especially susceptible to the phenotypic effects of an activated H-ras gene.

If tumor formation in these mice is relatively late and rare (as observed with other transgenic oncogene constructs), preweanling animals will be treated with carcinogens. Tumors from such mice will be very useful for investigations of secondary genetic events in hepatocarcinogenesis.

In contrast to most B6C3F$_1$ hepatomas, DNA from more than half of the N-nitrosodiethylamine-induced tumors examined previously were unable to morphologically transform NIH 3T3 cells. However, DNAs from three such hepatomas were capable of conferring the tumorigenic phenotype in nude mice to NIH 3T3 cells or to an immortal sup$^-$ Syrian hamster embryo cell line after cotransfection with a cosmid vector. Southern analyses of these nude mouse tumors showed the transforming genes were not members of the ras gene family and secondary transfections are under way to identify appropriate tumors for cloning these genes by the cosmid rescue procedure. This analysis should lead to the identification of novel genes which play a role in B6C3F$_1$ hepatocarcinogenesis.

Comparison of restriction fragment length polymorphisms (RFLP) in normal vs neoplastic tissue has frequently revealed a loss of heterozygosity in specific chromosomal regions. Such studies have provided strong evidence for the importance of tumor suppressor genes in various human cancers but this type of analysis has not been applied to experimental carcinogenesis models. The B6C3F$_1$ mouse is ideally suited for this type of analysis since it is a hybrid between two inbred mice for which RFLPs have been mapped to most chromosomes. In collaboration with Dr. J. Stone (Jackson Labs), DNAs from chemically induced B6C3F$_1$ hepatomas are currently being examined by Southern analysis with a human Rb-1 gene probe; this probe detects an Eco R1 polymorphism between the parental strains on chromosome 14. This study will be extended with additional RFLP probes for other mouse chromosomes in an attempt to identify a tumor suppressor gene which plays a role in B6C3F$_1$ hepatocarcinogenesis. If the transgenic lines described above develop hepatomas, they will provide an exciting new source of tumor tissue for RFLP studies. In addition, the RFLP analyses will be extended to other chemically induced and spontaneous tumors of normal and/or transgenic B6C3F$_1$ mice, i.e., lung and mammary adenocarcinomas and lymphomas.

In summary, the Laboratory of Molecular Carcinogenesis is studying the genes altered in cancers including tumor suppressor genes and oncogenes, the mechanisms by which chemical and other environmental substances act on these genes, and the basis for susceptibility of individuals to cancer causing substances. These studies will lead to new understandings of how environmental substances affect cancer and other human health problems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 25001-11 LMC

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Mutagenesis in Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.C. Barrett	Research Chemist	LMC	NIEHS
Others:	P. Lamb	Biologist	LMC	NIEHS
	J. Stowers	IRTA Fellow	LMC	NIEHS
	R. Wiseman	NRC Fellow	LMC	NIEHS

COOPERATING UNITS *(if any)*

Laboratory of Reproductive and Development Toxicology, DIR (Dr. J. McLachlan)
Mt. Sinai Hospital (Dr. N. Suzuki)
Nippon Dental University, Tokyo (Dr. T. Tsutsui)

LAB/BRANCH

Laboratory of Molecular Carcinogenesis

SECTION

Cellular Carcinogenesis

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Most chemical carcinogens induce DNA damage and are mutagenic at specific genetic loci; however, certain carcinogens (including the human carcinogens diethylstilbestrol, asbestos, arsenicals and benzene) usually do not induce gene mutations. We have examined the activity of these chemicals to induce morphological transformation, gene mutations and chromosome mutations in Syrian hamster embryo cells in culture. We have reported previously that diethylstilbestrol (DES) induces transformation in the absence of mutations at specific genetic loci. Furthermore, we have proposed that the mechanism of action of DES is related to its ability to induce numerical chromosome changes, i.e., aneuploidy. DES has colcemid-like activity and disrupts microtubule organization. Currently, DES-induced aneuploidy is being examined in the newborn mouse genital tract to test whether these changes occur in vivo in the target tissue. The mechanism of another important human carcinogen, asbestos, was also examined. The ability of asbestos and other mineral fibers to induce cell transformation depends on fiber dimension similar to the results in vivo for mesothelioma induction. We have proposed that asbestos induces cell transformation due to its ability to induce chromosomal changes. We have examined the role of proto-oncogene activation in human mesotheliomas, which are associated with asbestos exposure. DNAs from several mesotheliomas were positive for transforming activity in the NIH/3T3 nude mouse tumorigenicity assay. The transforming genes do not appear to be members of the ras gene family and we are currently cloning these genes. Arsenicals are effective inducers of cell transformation. Sodium arsenite and sodium arsenate are inactive as gene mutagens but are potent inducers of chromosome aberrations and also gene amplification. Benzene induces cell transformation but is a weak gene mutagen. This chemical is a very effective inducer of aneuploidy in this system. These results further support our hypothesis that cell transformation involves a chromosomal mutation and suggest an important role for carcinogen-induced aneuploidy in carcinogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 25029-04 LMC

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Neoplastic Transformation by Viral and Cellular Oncogenes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Tona Gilmer	Senior Staff Fellow	LMC	NIEHS
Others:	Bartel Turk	Biological Lab. Tech.	LMC	NIEHS

COOPERATING UNITS *(if any)*

University of Virginia (T. Parsons)

LAB/BRANCH

Laboratory of Molecular Carcinogenesis

SECTION

Cellular Carcinogenesis

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Tumor-derived Syrian hamster embryo (SHE) cell lines, induced *in vitro* by treatment with chemical carcinogens, contained increased levels of pp60$^{c\text{-}\underline{src}}$ kinase activity compared to preneoplastic parental cell lines and normal SHE cells. The increased kinase activity did not result from an increase in the pp60$^{c\text{-}\underline{src}}$ content of the SHE cell lines, but represented a 4-11 fold increase in pp60$^{c\text{-}\underline{src}}$ kinase specific activity. Both the extent of phosphorylation and the velocity of pp60$^{c\text{-}\underline{src}}$ phosphotransferase activity were increased in the tumor-derived cell lines. SHE cell lines producing chicken pp60$^{c\text{-}\underline{src}}$ were isolated following co-transfection with plasmids bearing the chicken c-*src* and neo^{r} genes. Chicken pp60$^{c\text{-}\underline{src}}$ expressed in an asbestos-transformed tumor-derived cell line showed an approximate 3-fold activation of tyrosine kinase activity compared to chicken pp60$^{c\text{-}\underline{src}}$ expressed in the preneoplastic cell line. We suggest that these results indicate that activation of pp60$^{c\text{-}\underline{src}}$ is mediated by *trans*-acting cellular factors present in the tumor-derived cells. Analysis of pp60$^{c\text{-}\underline{src}}$ in normal SHE cells, preneoplastic cell lines and tumor-derived cell lines showed no alteration in the phosphorylation of tyr-527 or tyr-416, two tyrosine residues whose phosphorylation states have been associated with modulation of kinase activity. In addition, a strong correlation was observed between the activation of endogenous pp60$^{c\text{-}\underline{src}}$ tyrosine kinase specific activity and the presence of additional phosphotyrosine-containing proteins. These studies indicate that the neoplastic progression of cells may be accompanied by the activation of proto-oncogene products, such as the pp60$^{c\text{-}\underline{src}}$ tyrosine kinase, by mechanisms that may not directly involve genetic alteration of the proto-oncogene DNA sequence and that novel tyrosine phosphorylations may result from this activation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 25031-02 LMC

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Tumor Suppressor Genes and Oncogenes in Chemical Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.C. Barrett	Research Chemist	LMC	NIEHS
Others:	O. Sugawara	Visiting Fellow	LMC	NIEHS
	P. Cizdziel	Staff Fellow	LMC	NIEHS
	H. Satoh	Visiting Fellow	LMC	NIEHS
	J. Boyd	Staff Fellow	LMC	NIEHS
	C. Jones & R. Whitehead	Q Appointments	LMC	NIEHS
	L. Annab	Biologist	LMC	NIEHS
	P. Lamb	Biologist	LMC	NIEHS

COOPERATING UNITS *(if any)*

Chemical Carcinogenesis Group, LMC
Kanagawa Cancer Center (Dr. M. Oshimura)
Cold Spring Harbor Laboratory (Dr. M. Lambert)

LAB/BRANCH

Laboratory of Molecular Carcinogenesis

SECTION

Cellular Carcinogenesis

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.5	5.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cancer development in humans and animals is a multistep process involving at least two classes of genes, proto-oncogenes and tumor suppressor genes. We have shown that neoplastic transformation of Syrian hamster embryo cells (SHE) in culture is a multistep process involving both activation of proto-oncogenes and inactivation of a tumor suppressor gene. Approximately 50% of the carcinogen-induced tumor cell lines had an activated H-ras oncogene, which was a late, post-immortalization step in the neoplastic progression of these cells. We have also shown that loss or inactivation of tumor suppressor genes is an essential step in the multistep neoplastic transformation of SHE cells. Nontumorigenic variants have been isolated which have lost (sup^{-}) or retained (sup^{+}) the ability to suppress tumorigenicity of tumor cells in cell hybrids. Fusions of sup^{+} or sup^{-} variants with different tumor cells show different patterns of suppression indicating that a family of tumor suppressor genes exists in these fibroblast cells. Currently, several strategies to clone tumor suppressor genes are in progress. A novel gene transfer assay has been developed using a cDNA library prepared from normal human fibroblasts in a mammalian expression vector, coupled with a highly efficient transfection procedure and a negative selection method. Another strategy involves cDNA libraries of sup^{+} hamster cells which have been screened with RNA from sup^{+} or sup^{-} cells and differentially expressed cDNAs have been cloned. Two-dimensional gel analyses of proteins have revealed a dramatic reduction in the expression of several tropomyosin isoforms which correlate with the loss of the tumor suppressor function. A cellular phenotype associated with the loss of tumor suppressor gene function has also been found. Sup^{-} cells suspended in agar respond reversibly to transforming and normal growth factors by forming colonies in agar whereas sup^{+} cells fail to grow. In addition to the tumor suppressor genes described above which are expressed in some immortal cell lines, tumorigenicity also may be limited by cellular senescence. Attempts to map the senescence phenotype to a particular human chromosome have suggested that human chromosome (#1) is involved.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 23000-01 LMC

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic Events in Hepatocarcinogenesis of B6C3F1 Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.W. Wiseman	NRC Fellow	LMC	NIEHS
Others:	E. Hou	Biologist	LMC	NIEHS
	J. Bisi	Biological Lab. Tech.	LMC	NIEHS

COOPERATING UNITS *(if any)*

Jackson Laboratory (J. Stone)

LAB/BRANCH

Laboratory of Molecular Carcinogenesis

SECTION

Chemical Carcinogenesis

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Liver tumors of the B6C3F$_1$ mouse, which arose spontaneously or were induced by a single dose of any one of 12 diverse chemical carcinogens at 12 days of age, have been examined for the activation of ras proto-oncogenes. To date, 141 such hepatomas have been screened and ~80% of them contained mutations in codon 61 of the H-ras proto-oncogene; another 5% had activated K-ras genes. The H-ras mutations in these hepatomas have been characterized by selective oligonucleotide hybridization or by the detection of new restriction sites which result from specific base substitutions in the 61st codon. The distinct patterns of ras mutations observed for each chemical indicated that these alterations are a direct result of the interaction of electrophilic ultimate carcinogens with these genes in vivo. These observations are also consistent with ras activation being an early event of hepatocarcinogenesis in B6C3F$_1$ mice. Diethylnitrosamine-induced hepatomas were exceptional since DNAs from 50% of these tumors were unable to morphologically transform NIH 3T3 cells. However, DNA from 3 such hepatomas was capable of conferring the tumorigenic phenotype in nude mice to an immortal nontumorigenic, Syrian hamster cell line. Attempts to clone these potentially novel non-ras transforming genes by cosmid rescue are in progress. The role of ras activation is also being examined by constructing transgenic C57BL/6N mice with germline activated H-ras genes for crosses with normal C3H/HeN mice. Tumor incidences in the parental line and hybrid offspring will be compared to determine whether the C3H/HeN genetic background is especially susceptible to the phenotypic effects of an activated H-ras gene. Finally, a panel of plasmid probes is being developed which detect restriction fragment length polymorphisms (RFLP) between C57BL/6N and C3H/HeN mice. These probes will be used to screen B6C3F$_1$ mouse tumors for losses of heterozygosity in specific chromosomal regions. Analogous studies have implicated tumor suppressor genes in a wide variety of human cancers.

LABORATORY OF MOLECULAR GENETICS

LABORATORY OF MOLECULAR GENETICS
Summary Statement

Mutagenesis is a critical component of carcinogenesis. It is also implicated in the aetiologies of other diseases with long latencies. In addition to these somatic cell events, germline mutagenesis has a public health impact that is difficult to ascertain or predict. The research in the Laboratory of Molecular Genetics concerns the origins, transmission and consequences of mutations.

The Laboratory of Molecular Genetics is comprised of two Sections. The research in the Mutagenesis Section focuses on the molecular biological mechanisms of DNA replication, DNA repair and mutagenesis. The research in the Gene Structure and Function Section addresses the impact of mutations on gene expression, development and populations. Model systems, ranging from bacteriophage to Drosophila and cultured mammalian cells, are utilized as efficient tools to learn about fundamental genetic mechanisms. The generalization and relevance of knowledge gained from such model systems to considerations of human health, especially environmental mutagenesis, is a historical fact and a continuing necessity. Many members of the Laboratory of Molecular Genetics are active as editors and reviewers and serve on NIEHS, NIH, national and international scientific committees. The training of junior scientists is an important component of the Laboratory of Molecular Genetics. About one half of the doctoral-level members of the Laboratory of Molecular Genetics are trainees. The ongoing success of the training component is reflected in the success of past trainees.

Mutagenesis Section

A major component of the NIEHS mission is to understand and prevent environmentally caused diseases that appear only long after an exposure. Heritable birth defects and cancer, and probably coronary heart disease as well, are such diseases. Because mutation plays an important role in their etiology, the aim of the Mutagenesis Section is to investigate how DNA damage causes mutations and lethality. The research is conducted by six independent but strongly interactive lead scientists, together with their individual support staffs of technical and professional assistants.

We use model systems (cultured mammalian cells, microbes such as yeasts, bacteria and viruses, and purely enzymatic systems) to investigate fundamental mechanisms of DNA replication, DNA damage, lethality and mutagenesis. The significance of these studies is twofold. First, a deep understanding of mechanisms may reveal ways in which mutagenesis can be prevented even when DNA damage does occur. Second, a study of the mutational consequences of different kinds of DNA damage will help in making regulatory decisions; on average, for instance, large deletions produce more serious consequences than do point mutations.

Most mutations arise by a process called error-prone repair (EPR) in which DNA damage stalls the DNA replication process. DNA synthesis can be induced to bypass the lesion, but in a poorly templated and thus highly mutagenic manner. The Drake group studies EPR in bacteriophage T4. They have recently described variants of the T4 DNA polymerase that change both the frequency and quality of EPR-dependent mutagenesis, sometimes enhancing it and sometimes almost abolishing it. These mutations directly implicate the polymerase in a crucial step of EPR and will be crucial in probing the enzymatic basis of mutagenic damage bypass.

Dr. James M. Clark, who joined the Section late last year, has discovered a remarkable reaction carried out by DNA polymerases: untemplated blunt-end addition. When all four dNTPs are provided, adenine is preferentially added. This reaction is likely to be important in unusual situations, such as chromosome termination and mutagenesis. Both prokaryotic and eukaryotic polymerases carry out the reaction.

The Kunkel group studies the fidelity of DNA replication using DNA polymerases from sources as diverse as bacterial viruses, mammalian cells and the AIDS virus. They have found the accuracy of DNA synthesis by a human DNA replication complex to be much more accurate than that by the purified DNA polymerase alone, showing that additional fidelity factors enhance polymerase accuracy in human DNA replication. A similar result has been found using yeast polymerases. The mammalian DNA polymerase gamma has been found to contain a proofreading 3'-exonuclease, in sharp distinction to previous reports. A model of misalignment mutagenesis to explain frameshift mutations in noniterated sequences has been tested and found to be robust. The fidelity of the AIDS virus reverse transcriptase has been examined because of the high variability of the virus in nature; while reverse transcriptases are generally error-prone, this one is especially so.

The Schaaper group studies mutagenesis both in *E. coli* and in DNA target molecules replicating in cell extracts, a useful system intermediate between enzymology and the intact cell. They have completed an extensive analysis of the kinds of mutations arising spontaneously in cells defective in the *mutHSL* postreplication mismatch repair system, thus discovering the kinds of mutations made by the replication apparatus before subsequent repair. Strong hints have been found that these mutations reflect not only replication errors, but spontaneous DNA damage as well. A mutant defective in the *mutD* copyediting step of DNA replication, which is tightly coupled to DNA synthesis, has been found to be additionally defective in mismatch repair, probably because the latter system becomes saturated by the high initial error frequency. Finally, the *mutT* gene, which has long been known to prevent mutations from A:T to C:G, has been shown to specifically prevent A:G mispairs.

The Sugino group studies DNA replication, recombination and repair in yeasts. They have recently discovered a yeast recombinase, an enzyme that catalyzes the exchange of DNA strands in recombination and repair, which has the unusual property of not requiring an energy source. Several more proteins required for DNA synthesis have been found and one of these turns out to be a protein kinase, suggesting that protein phosphorylation regulates the elongation step of DNA replication. They have achieved the first critical purification of the recently discovered yeast DNA polymerase III and have found it to resemble the mysterious mammalian delta polymerase.

The Tindall group studies mutagenesis in cultured mammalian cells. They have adapted the DNA polymerase chain reaction for the rapid analysis of mammalian mutations at the level of DNA sequence, greatly shortening the time and reducing the expense of gathering this kind of information. Mutations induced by the widely used antitumor agent Mitomycin C have been shown to consist of both point mutations and deletions, the latter predominating at higher doses and presenting a much greater genetic hazard.

During the year, members of the Mutagenesis Section were represented, usually as invited speakers, at every meeting of note in the mutagenesis arena. Thomas A. Kunkel served as Chairman of the Hans Falk Lecture Series. John W. Drake served as Chairman of the International Program Committee of the XVI International Congress of Genetics and as the Editor of Genetics. An externally proposed major reorganization of the Mutagenesis Section occupied much of the Section's lead scientists' time.

Eukaryotic Gene Structure and Function Section

The research of the three work groups of the Eukaryotic Gene Structure and Function Section of the Laboratory of Molecular Genetics focuses on three areas: genetic, molecular and developmental analysis of selected gene loci in Drosophila, the investigation of the role of transposons in hypermutability and chromosome rearrangement, and the investigation of naturally occurring molecular genetic variation and the mechanisms that shape it. Most members of the Section have active interest in more than one of these topics. Although the questions being investigated range from gene molecular structure to population genetics, the common use of the Drosophila system of genetics, molecular and developmental biology leads to considerable intellectual and practical cohesion and interaction in the Section. This common technical base also increases cross-support among work groups and overall productivity.

While the Mutagenesis Section investigates DNA metabolism and mutagenesis in prokaryotic, fungal and mammalian cell model systems, some members of the Eukaryotic Gene Structure and Function Section investigate germline mutagenesis in the Drosophila. As the mechanisms and products of mutagenesis become more precisely described at the molecular level, better understanding of the structure and developmental function of eukaryotic genes is required to predict the consequences of newly arising mutations. Three projects in the EGSFS pursue this kind of information through the identification, cloning and structural characterization of *Drosophila melanogaster* genes in which mutations have interesting and important developmental consequences. Mutations at the *suppressor of sable locus*, *su(s)*, suppress the expression of certain transposon-induced mutations at other loci. Dr. Voelker's group has now determined the DNA sequence and conceptual gene product of *su(s)*. Ongoing work is directed toward the developmental and cellular function of its product. The molecular mechanisms behind this suppression phenomenon are likely to be important in the pretranslational steps of gene expression.

Dr. Judd's group is investigating several aspects of regulation of *white* locus gene expression. The analysis of particular transpositions of the *white* locus that lose their pairing-dependent (*zeste* dependent), autonomous and developmentally clonal gene expression, will provide new insights into this unique gene regulation system. Dr. Abbott has studied the genetics of the *hid* locus. The *hid* gene product plays a critical function in the morphological rearrangement of several tissues during development. Ongoing work is aimed at the cloning and molecular analysis of the gene's structure and expression.

Dr. Langley's group is investigating how a retrovirus-like transposon, *tom*, causes dominant eye morphology mutations (*Om*) when it inserts at any 22 loci. Present studies continue to focus on the genetic and molecular analysis of alleles at a particular locus, *Om(1D)*, as a path to the mechanistic understanding of this unique hypermutability and developmental system.

Members of the Judd and Langley groups are collaborating on the investigation of recombination between mispaired transposons as a cause of spontaneous chromosomal rearrangements. The regulation of this mutational process and its potential role in the containment of transposon copy number are being investigated.

The most difficult step in the assessment of the risk in germline mutagenesis is in the extrapolation to the population level. Population genetic variation is an important consideration in assessing individual sensitivity to environmental mutagenesis and in the prediction of any impact of increased germline mutation on the population. The research in the Langley group seeks through the study of molecular population genetic variation in Drosophila to understand the forces that shape it. The relationship of molecular variation to levels of recombination, geographic subdivision and phenotype variation in gene expression are being determined for many regions of the Drosophila genome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60147-05 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Mechanisms of SOS-Mutagenesis in Escherichia coli

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. M. Schaaper	Visiting Scientist	LMG NIEHS
Others:	R. L. Dunn	Biologist	LMG NIEHS
	R. Cornacchio	Stay-In-School Employee	LMG NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	0.5	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The SOS system of Escherichia coli plays a crucial role in many aspects of mutagenesis in the organism. The system is not normally present in the cell but becomes induced upon blockage of DNA replication by DNA damage. Its induction entails the expression of a large number of new gene products, several of which are thought to interact with the process of DNA replication, rendering it error prone and producing mutations on both damaged and undamaged DNA. The evidence for the existence of these components rests largely on genetic experiments. However, the elucidation of the nature of these components and their mechanisms of action requires a more direct biochemical approach. We have designed an in vitro DNA replication system in which the existence of the error-prone replication components may be tested. The system uses the conversion of single-stranded bacteriophage M13 DNA into its double-stranded form (ss → RF conversion) by crude extracts derived from either normal or SOS-induced cells. After replication, the product DNA is transfected to produce intact bacteriophage. The accuracy of the in vitro replication step is then assayed by measuring the frequency of mutant phage before and after replication. The accuracy of DNA replication in crude extracts is extremely high and resembles the in vivo accuracy. Our early efforts have already demonstrated that an understanding of the accuracy of DNA replication in SOS-induced cells requires a full understanding of the factors that are involved in maintaining normal accuracy. This aspect is therefore pursued simultaneously. E. coli mutator and antimutator strains with known (or presumed) defects in the process of DNA replication are essential tools in these studies. Increased error rates of in vitro DNA replication have indeed been demonstrated using extracts of several E. coli mutators. In case of the mutT mutator our results have demonstrated the presence of a separate function for the prevention of A:G mispairs at the replication step.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60148-05 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Error-Prone Repair in Bacteriophage T4

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. W. Drake	Head, Mutagenesis Section	LMG NIEHS
Others:	D. C. Nguyen	Chemist	LMG NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.3	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mutagens contribute to the human burden of heritable birth defects and cancer and probably to heart disease as well. Most mutagens in most organisms act by triggering a process called error-prone repair (EPR). Such mutagens' primary action is to damage DNA in ways that block the progress of the DNA replication complex. EPR then facilitates damage bypass in a poorly templated (and therefore mutagenic) manner. Thus, the DNA polymerase is expected to play a crucial role in EPR. We have recently found that certain mutations of the bacteriophage T4 DNA polymerase gene can enhance or suppress EPR. The strongest enhancing mutation, hm (for hypermutable), also changes the specificity of EPR, preferentially increasing the frequency of base pair substitution mutations. Although hm has been difficult to map by conventional crosses, we have now assigned it to the DNA polymerase gene. We have also ascertained the effect on EPR of some previously described DNA polymerase mutations, one of which virtually abolishes EPR. Thus, the T4 DNA polymerase is a major determinant of the quantity and quality of EPR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 61022-07 LMG

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Population Genetics of Transposable Elements

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. H. Langley	Research Geneticist	LMG, NIEHS
Others:	E. A. Goode-Montgomery	Biologist	LMG, NIEHS
	G. M. Simmons	Staff Fellow	LMG, NIEHS
	W. H. Stephan	Visiting Associate	LMG, NIEHS
	B. H. Judd	Research Geneticist	LMG, NIEHS
	S. M. Huang	Biologist	LMG, NIEHS

COOPERATING UNITS *(if any)*
Dr. N. Kaplan and R. Hudson, Biometry and Risk Assessment Program
Dr. Brian Charlesworth, Department of Biology, University of Chicago

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Eukaryotic Gene Structure and Function Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The main goal of this project is to study the population biology of transposable genetic elements (parasites of the genome) using Drosophila as a model system in conjunction with quantitative theoretical analysis. During this period, the research has focused on two topics: 1) What is the primary mechanism containing the numbers of transposable elements? and 2) Is the evolutionary diversity observed between copies of elements at the DNA sequence level consistent with quantitative models of the dynamics of the elements in natural populations? Work over the last several years in this laboratory have led to the hypothesis that unequal crossing over is an important mechanism in the containment of transposable elements. The theoretical analysis of this model were examined. Ongoing research concerns the rate of occurrence of such events. A large number of spontaneous chromosomal deletions have been collected and the breakpoints are being characterized at the DNA sequence level to confirm the role of crossing over between transposable elements. The cloning and DNA sequencing of copies of the transposable element hobo from sampled individuals, populations and species is ongoing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61024-06 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic and Molecular Analysis of Suppressor-of-Sable Function in Drosophila

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. A. Voelker	Research Geneticist	LMG, NIEHS
Others:	J. F. Sterling	Biologist	LMG, NIEHS
	J. P. Graves	Biologist	LMG, NIEHS
	W. Gibson	Research Chemist	LMG, NIEHS
	S. S. Carpenter	Biological Aid (SIS)	LMG, NIEHS
	T. J. Maness	Biological Aid (SIS)	LMG, NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Eukaryotic Gene Structure and Function Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.4	1.0	4.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recent findings in Drosophila have shown that 1) a significant proportion of spontaneous mutations are caused by insertions of mobile genetic elements, and 2) in certain genetic suppressor systems, suppressible alleles are caused by insertions of specific mobile elements. We are investigating the molecular mechanism of action of one such suppressor system: recessive mutations at the suppressor-of-sable [su(s)] locus suppress recessive mutations at the vermilion (v) locus that are caused by insertions of the mobile elements 412. Current results suggest that this suppression occurs pretranslationally.

DNA sequences of su(s) have been cloned and are being characterized structurally and functionally. An 8 kb segment of genomic DNA that gives rise to a 5 kb polyA+ message has been sequenced. It contains three open reading frames that range in size from 700 to 1800 bases. cDNA clones have not yet been recovered, so the precise splicing pattern remains unknown. A "conceptual" protein produced by the 3 ORFs has no homology with any protein in the protein data bases. Portions of the cloned 25 kb of DNA have been reintroduced by P element mediated transformation and allow an identification of genetic function with messages produced by the region. A segment of DNA which is homologous with only the su(s) message rescues both the primary phenotype of suppression and a secondary phenotype of cold-sensitive male sterility. Antibodies raised against fusion proteins produced by portions of the above ORFs specifically recognize the s(s) portion of the fusion protein, but fail to detect any proteins in extracts from Drosophila embryos or adults.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 61030-05 LMG

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular analysis of the Om mutation in Drosophila ananassae

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. H. Langley	Research Geneticist	LMG, NIEHS
Others:	S. Tanda	Visiting Fellow, LG	LMG, NIEHS
	W. Quattlebaum	Biologist	LMG, NIEHS

COOPERATING UNITS *(if any)*
Drs. C. W. Hinton and M. Matsuda, Wooster College, Wooster, Ohio; K. Saigo, Kyushu University, Fukuoka, Japan; Y. Yobari, Tokyo Metropolitan University, Tokyo, Japan;

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Eukaryotic Gene Structure and Function Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.0	0.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Drosophila specie D. ananassae is characterized by hypermutability, meiotic male crossing over and a transposon, tom that specifically jumps to 22 sites in the genome cause dominant eye morphology mutations, Om. In order to understand how the tom element specifically jumps to Om loci and how the retrovirus-like element causes the defect in the development of the eye a particular locus, Om(1D), has been intensively studied. Over twenty mutations have been isolated and characterized at the genetic, cytogenetic and DNA level. Extensive cloning in the Om(1D) site has identified most of the tom insertions; however more cloning is ongoing. Additional molecular and genetic analysis has been carried out on other Om loci and the Som locus where many spontaneous dominant suppressors have arisen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61037-04 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of DNA Replication in Eucaryotes: Yeast as a Model System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Sugino	Visiting Scientist	LMG NIEHS
Others:	R. K. Hamatake	Senior Staff Fellow	LMG NIEHS
	S. L. Eberly	Staff Fellow	LMG NIEHS
	H. Hasegawa	Visiting Fellow	LMG NIEHS
	J. Nakao	Visiting Fellow	LMG NIEHS
	A. B. Clark	Biologist	LMG NIEHS
	T. Sugino	Guest Worker	LMG NIEHS

COOPERATING UNITS *(if any)* Lucy M. S. Chang, Prof. & Chairperson, Dept. of Biochem., The Uniformed Ser. Univ. of Health Sci., Bethesda, MD; Dr. L. H. Johnston, Group Leader, Lab. of Cell Prepagation, Nat. Inst. for Med. Res., London, England; Dr. P. Burger, Assoc. Prof., Dept. of Biochem., Washington Univ., St. L., MO

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.8	3.8	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An in vitro DNA replication system using yeast 2-µm and ARS (autonomously replicating sequences) plasmid DNAs, developed in this laboratory, has been used to investigate the mechanism of DNA replication in yeast. To identify and purify enzymes and components required for yeast chromosomal DNA replication, the crude extract system has been fractionated and reconstituted with the help of several temperature-sensitive chromosomal DNA replication mutants. To aid in overproducing and purifying such DNA replication proteins, the DBF1 and 2 genes (which are required for the elongation step of DNA replication) and the TS26 gene (required for the initiation of DNA replication) have been cloned, their nucleotide sequences determined, antibodies to them raised and their regulation studied. It was found that the DBF2 protein is a serine/threonine-specific protein kinase, suggesting that protein phosphorylation regulates elongation of DNA synthesis in yeast.

Finally, yeast DNA polymerases I, II, and III (The last being newly discovered in yeast crude extracts) have been purified to homogeneity for the first time and studied extensively. Using inhibitors and antibodies against each purified DNA polymerase, it has been established that each polymerase is unique and has a different function in yeast cells. In order to study these functions, the molecular cloning of DNA polymerases I, II and III is underway using either antibodies or the amino acid sequences of oligopeptides generated from the purified polymerases. In the meantime, mammalian PCNA/cyclin, which is under cell-cycle control and is a subunit of DNA polymerase δ, has been shown to stimulate the yeast DNA polymerase III reaction, suggesting that yeast DNA polymerase III is a homolog of mammalian DNA polymerase δ.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61039-04 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of DNA Recombination and Repair in the Yeast Saccharomyces cerevisiae

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, ~~laboratory, and institute affiliation~~)*

PI:	A. Sugino	Visiting Scientist	LMG NIEHS
Others:	C. C. Dykstra	Guest Worker (NRC Fellow)	LMG NIEHS
	A. B. Clark	Biologist	LMG NIEHS
	T. Sugino	Guest Worker	LMG NIEHS

COOPERATING UNITS *(if any)* Dr. M. A. Resnick, Res. Gen., TRTP, NIEHS, Res. Tri. Pk, NC Dr. F. E. Coleman-Wilson, Ass. Prof., Dept. of Microbio., Univ. of NC at Asheville, NC; Dr. K.-I. Arai, Dir., Dept. of Mol. Biol., DNAX Res. Inst., Palo Alto, CA

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An ATP-independent activity which catalyzes the transfer of one strand from a linear duplex DNA molecule to a complementary circular single strand has been detected in crude extracts from both mitotic and meiotic yeast cells. The assay requires the addition of yeast single-stranded DNA binding protein (ySSB). The polypeptide (yeast Strand Transfer Protein α, ySTPα) responsible for this activity has been purified to homogeneity from meiotic cells, characterized, and antibodies raised from a rabbit. Using the antibodies, the gene for ySTPα has been isolated, its nucleotide sequence determined, and its regulation studied. Although the gene is not essential for mitotic cell growth, it is required for meiotic homologous recombination and sporulation, proving that ySTPα is one of the meiotic recombination components in yeast and that the ATP-independent reactions catalyzed by ySTPα are biologically important. The ySTPα mRNA and polypeptide are constitutively expressed in both mitotic and meiotic yeast cells. However, the polypeptide is uniquely activated during meiosis by a mechanism that has not yet been identified.

In addition, one of the yeast DNA repair genes, RAD18, has been cloned, its nucleotide sequence has been determined, its polypeptide overproduced and purified, and its regulation studied.

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 61041-02 LMG

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular genetic variation in natural populations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Charles H. Langley	Research Geneticist	LMG, NIEHS
Others:	Naohiko Miyashita	Visiting Fellow	LMG, NIEHS
	Gail M. Simmons	Staff Fellow	LMG, NIEHS
	Wolfgang Stephan	Visiting Associate	LMG, NIEHS
	William Quattlebaum	Biologist	LMG, NIEHS
	Barbara Lange	Biologist	LMG, NIEHS

COOPERATING UNITS *(if any)*

Dr. Martin Kreitman, Department of Biology, Princeton University

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Eukaryotic Gene Structure and Function Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.75	3.0	1.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The primary focus of this project is the investigation of the relative roles of mutation, recombination, genetic drift and natural selection in shaping the levels of genetic variation observed at the DNA level. Specific experiments look for evidence of migration and population subdivision in the geographic distribution of DNA sequence variation at the *Adh* locus in *Drosophila melanogaster*. Several experiments address the fundamental question: what is the quantity and quality of molecular population genetic variation? To obtain a general answer many loci (*white*, *yellow* to *achete*, *g-6-phd*, *forked*, *vermilion*, *suppressor of forked* and *zeste*) in natural populations of Drosophila have been surveyed. A specific question in these and comparative studies with other species is the consequence of large differences in the amounts of crossing over per kilobase on the molecular genetic variation. The hypothesis that environmental heavy metals have selected for duplication of the metallothionein gene in natural populations is being tested by comparing the frequencies of these variants in locations with large differences in lead and cadmium pollution.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61042-02 LMG

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gene Expression in Early Development of Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael Abbott	Staff Fellow	LMG, NIEHS
Others:	Suzan Kazemi	Biological Aid (SIS)	LMG, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Eukaryotic Gene Structure and Function Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.0	.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The head involution defective (hid) locus is a previously uncharacterized gene located on the left arm of chromosome III of Drosophila melanogaster. At present, eleven recessive lethal mutations of this gene have been recovered and studied. These investigations reveal that the hid gene must be expressed sometime during the first 12 hours of embryogenesis so that the head of the fly larva will form correctly. In addition to its embryonic role, hid must also be expressed during the pupal stage to facilitate the construction of the adult fly. The role of the hid gene during embryogenesis may be related to its role later in development, that is, in both cases the hid product is required in specific groups of cells to permit these cells to undergo the proper rearrangements during development.

The genetic control of cell movement and tissue reorganization, two fundamental developmental processes, is poorly understood. Consequently, further studies on the role of the hid gene in Drosophila development may provide valuable insight into the genetic regulation of these processes and such knowledge would permit the construction of a model to guide future developmental investigations. At present, the cloning of hid DNA is in progress. The availability of the cloned DNA will permit: (1) a more precise determination of the timing and localization of hid expression during development, (2) eventually lead to the generation of monoclonal antibodies to the hid protein(s), and (3) facilitate attempts to identify other Drosophila genes which either are involved in the control of hid expression and/or share homologous DNA sequences which possess a common function in development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65034-04 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Specificity of Spontaneous and Induced Mutation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. M. Schaaper	Visiting Scientist	LMG NIEHS
Others:	R. L. Dunn	Biologist	LMG NIEHS
	R. Cornacchio	Stay-In-School Employee	LMG NIEHS

COOPERATING UNITS *(if any)*

R. P. Fuchs, Institut de Biologie Moleculaire et Cellulaire, Strasbourg, France
R. G. Fowler, San Jose State University, San Jose, CA

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	0.5	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In this project the mechanisms of mutagenesis are investigated through a detailed study of its specificity. DNA sequence information is gathered on all the classes of mutations that occur: base substitutions, frameshifts, deletions, duplications, insertion elements, complex rearrangements, etc. These classes have their own dependence on the local DNA sequence and generally result from different mutational pathways. The specificity of mutation thus provides a way to analyze and separate the various components of mutation. We use the *lacI* gene of the bacterium *E. coli* as a mutational target. The gene codes for the repressor of the *lac* operon and forward mutations to $lacI^-$ are scored based on their constitutive expression of the operon. The $lacI^-$ genes (typically several hundreds at a time) are transferred by *in-vivo* recombination to a single-stranded (recombinant) phage vector and sequenced, producing the mutational spectrum of interest. Comparing spectra in strains affected in various DNA repair/replication pathways is a next important step. In case of defined enzymatic pathways, the spectra provide direct direct correlations between mutational classes and their responsible pathways. In case of unknown pathways, the mutational specificity may provide new insights into the affected pathway. So far, we have determined the specificity of mutation in *mutH*, *mutL*, *mutS*, *mutT* and *mutD* and wild-type strains of *E. coli* and have gained insights into the specific contributions of DNA damage, DNA mismatch repair and exonucleolytic proofreading to mutation. In case of induced mutagenesis, the specificity of mutation is a tool to identify both the nature of the premutagenic lesions and the mechanisms by which these are converted into mutations. Recent examples of this approach are the determination of the specificity of mutagenesis by ultraviolet light and the chemical carcinogen N-acetoxyacetylaminofluorene (NAAAF).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65036-04 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gene Organization and Regulation in D. melanogaster

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	B. H. Judd	Head, EGSFS	LMG, NIEHS
Others:	Patricia S. Davis	Chemist	LMG, NIEHS
	Shu-Mei Huang	Biologist	LMG, NIEHS
	Katherine M. Peterson	P Appointment	LMG, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Eukaryotic Gene Structure and Function Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.50	0.25	2.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to understand more fully the mechanisms of gene regulation during eukaryotic development. The approach is to study selected loci having mutations that perturb regulatory functions. One system of interest involves the interaction of the zeste and white loci in Drosophila melanogaster. The mutation of z^1 represses normal white locus (w^+) function to give yellow eye color, but only if there are two w^+ genes that are paired or proximate. This position-dependent regulatory phenomenon is called transvection. We are studying mutations of white locus that interact with z^1 in ways that modify the transvection effect. For example, w^{zm} gives a mottled-eye color in a single dose, but only in a z^1 background; otherwise it is wild type. The w^{zm} mutation resulted from the insertion of the retrovirus-like transposon BEL in the 5′-most intron of the white locus, very near the sites where the zeste protein is known to bind. w^{zm} is mildly unstable and has produced a series of other alleles including a transposition of an X chromosome segment containing white into the third chromosome. In the new position, the white locus produces a wild-type phenotype except when the transposition is homozygous and the z^1 mutation is present. The transposition produces a mosaic eye-color that is nonautonomous and nonclonal in expression, marking a dramatic change from the autonomous, clonal patterns seen in the non-transposed w^{zm} and its derivatives. The molecular analyses of these mutant alleles are being done to determine the basis for the modified expression of white and its interaction with zeste.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65037-04 LMG

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Transposon - mediated chromosome instabilities in Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	B. H. Judd	Head, EGSFS	LMG, NIEHS
Others:	Shu-Mei Huang	Biologist	LMG, NIEHS
	C. H. Langley	Research Geneticist	LMG, NIEHS
	E. A. Goode-Montgomery	Biologist	LMG, NIEHS

COOPERATING UNITS *(if any)*

Dr. Johng K. Lim, Distinguished Professor of Biology
University of Wisconsin, Eau Claire

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Eukaryotic Gene Structure and Function Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is focused on the role of transposons in the process of spontaneous mutation and chromosome rearrangement in Drosophila. With Prof. Lim we are studying a strain that showed a burst of activity by the retrotransposon gypsy, exhibiting amplified copy number and high mobility. This caused a high rate of spontaneous X chromosome mutation due to insertion/excision events. The mobilization is shown to occur very early after fertilization, causing somatic and germline mosaicism for mutations. We are studying the conditions that activate gypsy and also how an active strain becomes stable.

Transposons are also known to mediate gross chromosomal rearrangements through a process of asymmetrical pairing and exchange. This process occurs both as intrachromosomal and interchromosomal exchanges to produce, in the former case, deletions or inversions depending on the relative orientations of the transposons and in the latter case, duplications and deficiencies and possibly translocations. We are studying a large collection of such rearrangements and characterizing the breakpoints at the molecular level to establish the role of the transposons and investigate the mechanism of exchange.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65038-03 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Mechanisms of Mutagenesis by Animal Cell DNA Polymerases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. A. Kunkel	Research Geneticist	LMG NIEHS
Others:	A. Soni	Biologist	LMG NIEHS
	M. P. Smith	Biologist	LMG NIEHS

COOPERATING UNITS *(if any)*

Myron F. Goodman, University of Southern California, Los Angeles, CA
Robert A. Bambara, University of Rochester, Rochester, NY
Dale W. Mosbaugh, University of Texas, Austin, TX

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.5	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Replication and maintenance of the stability of genetic information requires the accurate synthesis of DNA. In animal cells, DNA synthesis is performed by four distinct classes of DNA polymerases, α, β, δ and γ. Our objective has been to characterize the accuracy of DNA synthesis by each of these enzymes and to analyze the errors committed by each in an attempt to understand how mutation rates are controlled.

A major effort this year has been to determine if the high fidelity of chick embryo DNA polymerase γ results from exonucleolytic proofreading of polymerization errors. This has led to the discovery of a new 3' → 5' exonuclease that proofreads so efficiently that this avian polymerase is one of the most accurate DNA polymerases describe to date. This establishes that at least two of the four classes of higher eukaryotic DNA polymerases achieve high fidelity by a proofreading mechanism. We intend to extend these polymerase-γ studies to mammalian organisms.

A second major effort has been to determine the mechanisms by which eukaryotic DNA polymerases discriminate between correct and incorrect nucleotides during polymerization. These efforts have focused on an analysis of the classical miscoding mechanism as well as on base substitution and frameshift errors resulting from misalignment mechanisms. For this purpose, we have just initiated steady state kinetic analyses of misincorporation to determine if the polymerases have active sites that change conformation to reject errors or if error rates are determined primarily by simple nucleotide dissociation rates. Eventually we want to extend these studies to complex enzyme systems that contain additional fidelity components.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65041-02 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

DNA Repair in Mammalian Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. M. Clark Senior Staff Fellow LMG NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mutagenic agents generally exert their effects by introducing structural lesions into DNA that block DNA replication. Error-prone repair, the major mechanism of damage-induced mutagenesis, occurs when DNA replication bypasses such damaged bases in a poorly-templated (and thus highly mutagenic) manner. Studies are currently underway in this laboratory to investigate this process in a mammalian system. A particular type of lesion will be introduced quantitatively into a DNA substrate at a single site. Its effects on DNA synthesis can then be assessed either by using purified mammalian replication proteins (the SV40 *in vitro* replication system) or by introducing the lesion-containing DNA into mammalian cells. By introducing the lesion into a gene (β-galactosidase) at a site where mutations are readily detectable, the mutagenic consequences of the lesion can also be evaluated.

Recently, a novel, non-templated nucleotide addition reaction was observed with DNA polymerase I (Klenow fragment) from *E. coli*. This reaction has now been demonstrated with enzymes from both procaryotic and eucaryotic sources and may be a common property of DNA polymerases. Non-templated DNA synthesis is important *in vivo* to replicate the ends of linear chromosomes (telomeres) and to generate antibody diversity. Reactions of this type may also represent a new mechanism to generate spontaneous mutations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65042-02 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Gene uvsW in Error-Prone Repair by Bacteriophage T4

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. W. Drake	Head, Mutagenesis Section	LMG NIEHS
Others:	L. K. Derr	Guest Worker	LMG NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS: 0.05	PROFESSIONAL: 0.05	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mutagens contribute to the human burden of heritable birth defects and cancer and probably to heart disease as well. Most mutagens in most organisms act by triggering a process called error-prone repair (EPR). Such mutagens' primary action is to damage DNA in ways that block the progress of the DNA replication complex. EPR then facilitates damage bypass in a poorly templated (and therefore mutagenic) manner. uvsW is a cruical but mysterious gene in the bacteriophage T4 EPR system. Mutations in uvsW depress recombination, increase killing and abolish mutagenesis by agents acting through EPR. Temperature-sensitive mutations of uvsW have been generated and characterized by mapping and complementation tests and their effects on survival, recombination and mutagenesis have been determined. A deletion mutations of uvsW has been engineered, providing a rigorously defined null allele. The expression and regulation of uvsW is being explored by a combination of DNA-sequencing, northern-blot, primer-runnoff and RNA-sequencing methods.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65043-02 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Gene uvsX in Error-Prone Repair by Bacteriophage T4

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. W. Drake	Head, Mutagenesis Section	LMG NIEHS
Others:	M. O. Rosario	IRTA Fellow	LMG NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.05	1.05	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mutagens contribute to the human burden of heritable birth defects and cancer and probably to heart disease as well. Most mutagens in most organisms act by triggering a process called error-prone repair (EPR). Such mutagens' primary action is to damage DNA in ways that block the progress of the DNA replication complex. EPR then facilitates damage bypass in a poorly templated (and therefore mutagenic) manner. The bacteriophage T4 uvsX gene plays a central role in EPR and also in recombination. Its product is a recombinase, a protein that catalyzes homologous strand exchange between DNA molecules. The specific role of this protein in EPR remains mysterious. Two analyses are underway. First, although several severe mutations of uvsX are only semilethal, there are hints that an even more drastic disruption of uvsX may be fully lethal. Therefore, mutations are being introduced into early parts of the gene and the resulting mutants are being examined for phenotype, including viability. Second, tests are being performed for a correlation between recombination and mutagenesis: in a cross employing outside markers, newly induced mutations are screened for locally enhanced frequencies of recombination.

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65044-02 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Replication Repair in Bacteriophage T4

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. W. Drake	Head, Mutagenesis Section	LMG NIEHS
Others:	D. C. Nguyen	Chemist	LMG NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mutagens contribute to the human burden of heritable birth defects and cancer and probably to heart disease as well. Most of the mutagenic and lethal effects of DNA-damaging agents are prevented by DNA repair systems. The classically described systems repair damage before DNA replication (by direct reversal or by excision repair), at the replication fork (by mutagenic bypass of damaged bases) or after replication (by a recombinational process). We have recently described a new mode of repair operating in bacteriophage T4. It is defined by several mutations that enhance killing by DNA-damaging agents. These mutations reside in vital genes encoding enzymes of DNA replication, and repair appears to occur during replication itself. The repair system is accurate (nonmutagenic). To date, mutations defective in replication repair have been found in the genes encoding the DNA helicase and the SSB protein (which binds single-stranded DNA, holding it in an extended configuration suitable for DNA replication). Attempts are underway to find further mutations affecting this repair process by screening the genes encoding T4 DNA primase and DNA polymerase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65045-02 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Bacteriophage T4 *rI* Mutations

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. W. Drake	Head, Mutagenesis Section	LMG NIEHS
Others:	D. C. Nguyen	Chemist	LMG NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.35	0.05	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mutagens contribute to the human burden of heritable birth defects and cancer and probably to heart disease as well. Bacteriophage T4 has been widely employed as a model system to analyze mechanisms of mutagenesis. One of the most common T4 mutation assays recognizes *r* (rapid lysis) mutants by their large, sharply edged plaques. Although the *rII* mutants are those most often subjected to further analysis, most mutagens produce more *rI* than *rII* mutants. Since little is known about the *rI* mutants, we have investigated their genetic properties. Mutations that produce the characteristic *rI* phenotype arise at two loci, one the classically described locus at about 1:30 on the standard map and another a locus at about 8:30. Point mutations at the 1:30 locus recombine *inter se* at low frequencies, suggesting a small gene; several are suppressed by unlinked but as yet unmapped suppressor mutations. The "8:30" locus is being more closely mapped.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65046-02 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Accuracy of DNA Replication in vitro

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. A. Kunkel	Research Geneticist	LMG NIEHS
Others:	J. D. Roberts	Senior Staff Fellow	LMG NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are interested in determining the mechanisms by which human cells control spontaneous and induced mutation rates. We have previously shown that the major replicative DNA polymerase in human cells, when purified as a relatively simple DNA polymerase of limited complexity, is not accurate enough during DNA synthesis reactions in vitro to account for low spontaneous mutation rates in vivo. However, it is well established that replication involves the concerted action of a number of proteins. We are therefore examining the fidelity of semiconservative bidirectional DNA replication by a human HeLa cell replication complex. Our initial results, obtained in a forward mutation assay capable of detecting a variety of errors, indicated that this replication system was at least 20-fold more accurate than the purified replicative DNA polymerase alone. This observation has been substantiated by several control experiments, necessitated by the complicated nature of the system. In order to more accurately assess the fidelity of replication, mutagenesis vectors have been constructed and used to monitor the base substitution and frameshift fidelity of the replication complex in highly sensitive reversion assays. Initial results with these assays demonstrate that for minus-one base frameshift errors at reiterated template sequences, DNA synthesis by the replication complex is at least 500-fold more accurate than purified DNA polymerase alpha. However, the base substitution fidelity of the replication complex, while high, is still insufficient to explain the extremely low mutation rates observed in intact cells. This suggests that additional mechanisms for maintaining accurate DNA synthesis are present within the cell. We are currently dissecting the replication system into its component parts and testing the effects of individual subunits on specific types of mutation events using the highly sensitive reversion assays. We intend to focus on mechanisms that contribute to fidelity, including base selectivity, exonucleolytic proofreading and post-replication error correction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65047-02 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Fidelity of Retroviral Reverse Transcriptases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T. A. Kunkel	Research Geneticist	LMG NIEHS
Others:	J. D. Roberts	Senior Staff Fellow	LMG NIEHS
	K. Bebenek	Visiting Fellow	LMG NIEHS

COOPERATING UNITS (if any)

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Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A critical feature of the life cycle of retroviruses, including the human immunodeficiency virus (HIV), is their ability to generate diversity. Retroviruses have high mutation rates, permitting rapid evolution of new forms of the virus that are better able to escape host defense mechanisms. A major source of this diversity may be the infidelity of the viral reverse transcriptase (RT). Our objective is to examine the mutagenic potential of RTs during DNA synthesis in vitro, using an M13mp2 mutagenesis assay that monitors a wide variety of base substitution, frameshift, deletion and complex errors. We began by measuring the error frequency of a single round of natural DNA synthesis with RTs isolated from avian myeloblastosis virus (AMV) and murine leukemia virus (MLV). Both enzymes exhibit low fidelity. DNA sequence analyses of mutants generated by the AMV RT demonstrate that it commits many different types of errors. Two specificities are unique; base substitution errors result exclusively from misinsertion of purine nucleotides and many -1 deletions occur at non-reiterated base sequences. Furthermore, in an assay employing a terminally mismatched template-primer, both AMV and MLV RTs exhibit an unexpected extension preference that is unique compared to normal cellular polymerases. These specificities may reflect a structural differences in the active site of RTs. Overall, these data support the concept that the observed high mutation rate of retroviruses indeed reflects low fidelity of reverse transcription. We have recently obtained a preparation of reverse transcriptase from HIV-1. Our initial results indicate that this enzyme is even more error prone than the AMV and MLV RTs. We are presently sequencing a large collection of HIV RT-generated mutants to determine the most common mutational events perpetrated by this enzyme. We also intend to use reversion assays to examine specific mutational pathways in order to elucidate the mechanisms by which the diversity of the HIV is generated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 65048-02 LMG

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Engineering DNA Polymerases to Probe Mutational Mechanisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. A. Kunkel	Research Geneticist	LMG NIEHS
Others:	K. Bebenek	Visiting Fellow	LMG NIEHS
	M. P. Smith	Biologist	LMG NIEHS

COOPERATING UNITS *(if any)*
Catherine M. Joyce, Yale University Medical School, New Haven, CT

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Laboratory of Molecular Genetics

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INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are interested in elucidating the mechanisms used by DNA polymerases to increase the accuracy of DNA synthesis. In both prokaryotes and eukayotes, the fidelity of DNA synthesis results from at least two discrimination steps. Errors are prevented as the phosphodiester bond is formed due to the base selectivity of the polymerization reaction, and if errors are committed at the first step they can be corrected by 3' → 5' exonucleolytic proofreading. We are using the large (Klenow) fragment of E. coli DNA polymerase 1 as a model protein to dissect these two steps. The cloning and overproduction (by C. M. Joyce at Yale) of the enzymatically active Klenow polymerase and the recent determination of its structure by X-ray crystallography provided data which enabled engineering of the protein by site-directed mutagenesis. Two mutant derivatives of the Klenow polymerase, each lacking the proofreading exonuclease activity but containing normal protein structure, and a third protein containing only the polymerase domain, have been constructed. We have compared the fidelity of these proteins to that of wild-type Klenow polymerase to determine the contribution of base selectivity and proofreading to fidelity. These data demonstrate that the base selectivity of polymerization achieves an error rate of 1/10,000, and that proofreading improves fidelity about 5-fold overall. There are wide variations in discrimination at both steps, depending on the template site and error. Measurement of the steady state kinetic constants for correct and incorrrect incorporation events suggest that base selectivity depends on both the ability of the polymerase to reject incorrect bases by changing active site conformation and on differences in the amount of time a nucleotide will occupy the active site based on hydrogen bonding potential and base stacking interactions. Preliminary results with the polymerase domain suggest that, independent of proofreading, the fidelity of polymerization is influenced by the small exonuclease domain, particularly for deletion errors. These studies are intended to describe the relationships between enzyme structure and the production of mutations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65049-02 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Mutagenesis With Yeast Replication and Repair Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. A. Kunkel	Research Geneticist	LMG NIEHS
Others:	A. Sugino	Visiting Scientist	LMG NIEHS
	R. K. Hamatake	Senior Staff Fellow	LMG NIEHS
	M. P. Smith	Biologist	LMG NIEHS

COOPERATING UNITS *(if any)*

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Mutagenesis Section

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.1	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The simple eukaryote Saccharomyces cerevisiae provides a good genetic system and a source of highly purified DNA replication and repair proteins for the study of molecular mechanisms of mutagenesis. A major effort of the past two years has been to determine the fidelity of DNA synthesis by the major replicative polymerase in yeast, DNA polymerase I. To examine the possibility that subunits other than the polymerase catalytic subunit may influence fidelity, we measured the accuracy of yPolI purified by conventional procedures, which yields polymerase with a 140 Kd catalytic subunit and no associated primase activity, and that of yPolI purified by immunoaffinity chormatography, which yields polymerase having a catalytic subunit of ~180 Kd as well as three additional polypeptides and primase activity. The average fidelity was similar for both forms of polymerase and demonstrate that, like its equivalent in higher eukaryotes (polymerase α), both yPolI and the polymerase I-primase complex produce three predominant classes of errors, single-base substitutions, single-base frameshifts and larger deletions. For specific errors and template positions, the two forms of polymerase do exhibit interesting fidelity differences. Despite these differences, when considering the overall error frequency and the spectrum of DNA synthesis errors, the results suggest that the polymerase I-primase complex is not highly accurate, and, just as for the polymerase alone, its fidelity is not sufficient to account for low spontaneous mutation rates in vivo. The mutant specificity data also suggest models to explain two subsets of errors. First, we propose that in the appropriate sequence context certain base misinsertion events can rearrange to form stable misalignments that lead to -1 frameshifts even in non-reiterated sequences. Results of DNA polymerase reactions with specifically designed mismatched template-primers support this model. Second, among the deletions, many of which are between direct repeats, is a subset that contain unusual junctions. These can be explained by aberrant "loop-back" synthesis by yPolI to generate complex deletions by a direct repeat mechanism.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65050-02 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Analysis of Deletion Mutations in Chinese Hamster Ovary Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	K. R. Tindall	Staff Fellow	LMG NIEHS
Others:	P. S. Alexander	Biol. Lab. Tech.	LMG NIEHS
	D. H. Halderman	Biol. Lab. Tech.	LMG NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 0.3	OTHER: 0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Deletion mutations and chromosome rearrangements have been implicated in a variety of human genetic defects including oncogene activation. Little is known regarding the mechanisms by which genomic rearrangements arise, largely due to the lack of convenient systems for the study of such events. The Chinese hamster overy (CHO) cell line, AS52, has been demonstrated to be particularly useful in the study of deletion mutations. AS52 cells were constructed by transfecting a single copy of the bacterial gpt gene into a hprt-deficient CHO host and isolating a cell line that carries gpt stably integrated into the CHO genome. AS52 cells appear to be capable of detecting mutations induced by clastogens or radiomimetic agents while analogous loci such as hprt show little or no mutational response to many of these agents. However, classical point mutagens such as UV, EMS, ICR-191 induce mutations at either loci with approximately equal efficiency. These results are thought to reflect the nature of the gpt integration site. We propose that AS52 cells allow the recovery of viable multilocus deletions i.e., deletions affecting all or part of the gpt locus and adjacent essential DNA sequences) whereas multilocus deletions will be conditionally lethal at the hemizygous X-linked hprt locus. One mutagen of interest is the potent antitumor agent, mitomycin C(MMC). At lower doses, MMC induces mostly point mutations as determined by Southern blot analysis. At higher doses of MMC, a high frequency of deletion mutations is induced. These data represent the first molecular demonstration of a change in mutational spectrum with a change in mutagen dose in mammalian cells. We are currently in the process of analyzing the MMC-induced point mutations using DNA sequence analysis and we are using the πvx-recombinational recovery system to isolate deletion endpoints from genomic lambda libraries derived from selected spontaneous and MMC-induced deletion mutants. The ability to recover deletion mutants as viable clones using the AS52 cell line allows detailed studies of the mechanistic pathways by which deletion events occur in the mammalian genome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65051-02 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Analysis of Point Mutations in Chinese Hamster Ovary Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	K. R. Tindall	Staff Fellow	LMG NIEHS
Others:	B. P. Holway	Guest Worker/Summer Grad	LMG NIEHS

COOPERATING UNITS (if any)

Dr. Leon F. Stankowski, Jr., Pharmakon Research International, Inc., Waverly, PA

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have continued our studies of point mutational changes in mammalian cells using the AS52 cell line. AS52 cells carry and express (using the SV40 early promotor) a single copy of the bacterial gpt gene stably integrated into the genome of hprt-deficient Chinese hamster ovary (CHO) cells. The gpt gene is analogous to hprt and one can readily isolate 6-thioguanine resistant (6TG^r) mutant AS52 cells. We have begun a detailed characterization of spontaneous mutations in AS52 cells. In two separate experiments, independent spontaneous 6TG^r AS52 mutants were characterized by Southern blot analysis. Both surveys indicate that approximately 60% of the spontaneous mutants are deletions or rearrangements of the gpt sequences. The remaining 40% show no alteration from the Southern blot pattern of the nonmutant parental AS52 cell line and have been designated putative gpt point mutations. We have simplified the process of AS52 mutant sequence analysis by using the polymerase chain reaction (PCR) technique to amplify spontaneous gpt mutant sequences in vitro using the Taq DNA polymerase. We have measured the replication fidelity of the Taq polymerase. This relatively error-prone enzyme yields an average of 1 base substitution per 9000 nucleotides synthesized and an average of 1 frameshift mutation per 40,000 nucleotides synthesized. In addition, we have developed techniques to allow for the direct DNA sequence analysis of the amplified PCR product, eliminating the effect of any mutations that occurred during PCR. Thus, any mutation observed must have been present in the initial mutant colony. Results thus far indicate that most spontaneous mutations are small deletions, although a limited number of base pair substitutions have been observed. Notably, there is a 3-base deletion hot spot in the gpt gene that has been observed in approximately 50% of the gpt mutants sequenced. We intend to continue to use PCR and direct DNA sequence analysis to generate mammalian point mutational spectra derived as a result of induced mutations in AS52 cells. The first induced spectrum to be analyzed will be point mutations occurring at lower treatment doses of the anti-tumor agent and mutagen, mitomycin C.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65052-02 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Use of Retroviral Vectors in the Analysis of Mutations in Mammalian Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. R. Tindall	Staff Fellow	LMG NIEHS
Others:	B. P. Holway	Guest Worker/Summer Grad	LMG NIEHS

COOPERATING UNITS *(if any)*

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Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.2	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are using retroviral vectors to construct cell lines useful for the study of mutational mechanisms in human cells. Under appropriate infection conditions one can isolate single copy genomic integrations of retroviral vectors, a requirement for mutagenesis studies. The retroviral vectors used in these studies carry and express both the bacterial gpt and neo genes. An appropriate integration of the gpt gene and the linked neo gene provides cellular resistance to both mycophenolic acid and the aminoglycoside, G418. We have evaluated several vector constructions to determine requirements for the optimal expression of both the gpt and neo genes. We find optimal expression in constructions in which the gpt gene is under the regulatory control of the SV40 promoter and the neo gene is under the regulatory control of the 5'-LTR. We are also constructing vectors that allow differential regulation of the gpt gene using the metallothionein promoter. Using these vectors, we are in the process of generating single copy gpt integrations into the genome of the human cell line, HT1080. HT1080 cells are derived from a human fibrosarcoma and have a particularly stable karyotype, another important requirement for mutagenesis studies. Our goal is to determine the effect of genomic position on both spontaneous and induced mutations at a chromosomally integrated gpt locus in human HT1080 cells. Mutant spectra will be generated for comparative analysis from various HT1080 derived independent gpt integrations. Such an approach provides a unique opportunity to evaluate "position effects" on mutagenesis using the same target gene (i.e., gpt) integrated at various sites in the genome. Long term, these studies should provide a data base for the use of retroviral vectors in the construction of human repair-deficient cell lines useful for mechanistic studies of mutations.

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65053-01 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

DNA Sequence Characterization of Bacteriophage T4 rII Mutations

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. W. Drake	Head, Mutagenesis Section	LMG NIEHS
Others:	M. C. Kricker	Staff Fellow	LMG NIEHS

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NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.05	1.05	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are using the bacteriophage T4 rII system as a model to explore mechanisms of DNA damage and mutagenesis. Because traditional DNA sequencing methods for analyzing the molecular nature of rII mutations are laborious and slow, we are developing methods based on genomic sequencing and/or the polymerase-chain-reaction method of sequence amplification. Then, important classes of mutations will be examined for their sequence changes. For instance, even mild heat damages DNA and could, if uncountered by repair, produce on the order of 100 mutations per diploid human cell per day. Earlier studies showed that heat induces both transitions and transversions at G:C base pairs in phage T4. Genetic studies suggested that the main heat-induced transversion pathway is G:C → C:G but did not exclude G:C → A:T, and the distinction can be easily resolved by sequencing studies.

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65054-01 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Invariant Per-Genome Mutation Rates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. W. Drake Head, Mutgenesis Section LMG NIEHS

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NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In the late 1960s the then-available data suggested that a variety of microbes exhibited a constant forward mutation rate of about 0.003 per genome per replication; genome sizes varied by about 1000-fold, and so, inversely, did mutation rates per base pair per replication. This observation suggested that mutation rates had evolved to an optimum that was surprisingly constant among diverse organisms. Since then, the data base has improved for many organisms and the invariance of per-genome mutation rates appears not only still to be the norm, but to extend all the way from a DNA-containing bacteriophage to a lower multicellular eukaryote. (Some RNA viruses and DNA plasmids constitute the presently known exceptions.) This data base is being reexamined and analyzed to test the generality of the relationship.

LABORATORY OF MOLECULAR AND INTEGRATIVE NEUROSCIENCE

LABORATORY OF MOLECULAR AND INTEGRATIVE NEUROSCIENCE

SUMMARY STATEMENT

The Laboratory of Molecular and Integrative Neuroscience is the central focus within NIEHS for studies concerning the environment and the brain. The primary goal of this laboratory is to study the basic mechanisms mediating brain regulation of neuroendocrine, cognitive, behavioral, and reproductive functions and to elucidate the complex interactions between the brain and the environment that result in normal or abnormal neural activity. The subjects of cell communication, neuroendocrine regulation and signal transduction are explored using modern molecular and cell biology techniques. The integrative role of neuropeptides in regulating central and peripheral events is a central theme, offering opportunities for an interdisciplinary, multifaceted approach. The Laboratory presently is organized into five sections or workgroups. Those groups as well as their areas of research and recent accomplishments are described below:

Scientists in the Reproductive Neuroendocrinology Section study the basic mechanisms regulating the activity of the "neuroendocrine brain," i.e., those neurons and neuronal circuits within the brain involved in the control of endocrine, autonomic, growth, reproductive and behavioral functions. Specialized peptidergic neuronal systems are the key regulators of these functions, and the peptide signals emitted from these neurons represent the primary messengers of the regulatory cascade. A major advance towards understanding this process of cell communication came with the realization that secretion of these peptides occurs in a pulsatile pattern, which is essential for the neuronal-peripheral cell communication to be effective. Recent studies evaluated the role of different intracellular messenger pathways leading to activation of the arachidonic acid cascade and/or protein kinase C activation in the secretion of LHRH from the hypothalamus. It was observed that arachidonic acid metabolized via lipoxygenase gives rise to an inhibitory, as yet unidentified product. This putative lipoxygenase metabolite may play a very important role in providing signals to turn off the neuron after its initial activation, a pattern of activity essential for the generation of secretory pulses. This is also relevant to recent findings from this group which indicate that, in a truly autocrine fashion, LHRH neurons can turn off their own activity by a mechanism that probably involves recurrent collaterals and LHRH autoreceptors. In addition, this group has conducted a detailed characterization of the pattern of, and the role of steroids on, pulsatile LHRH secretion into the portal-microvasculature. This work has led to the novel concept that, contrary to dogma, gonadal steroids actually enhance, rather than inhibit, secretion of the neuropeptide, an observation supported by studies on the effects of steroids on LHRH mRNA expression.

To understand the role of pulsatility on activation of cellular responses, a computerized perifusion system designed to mimic the in vivo "pulse generator" has been developed. Patterns of pulses recorded in vivo are duplicated in a computerized input and the cellular responses analyzed kinetically to obtain information about the encoding of signals in the pulsatility pattern and also about the involvement of intracellular messengers on the decoding process at the cellular level. These studies are correlated with in vivo models in which the pattern of secretion of different pituitary hormones (LH, FSH, Prolactin, ACTH) are evaluated and further analyzed using a combination of immunoneutralization and agonist replacement therapy. The latter approach led to the important discovery that FSH pulses are independent of LHRH input and to a renewed interest in the search for an FSH releasing factor.

Researchers within the Neuropharmacology Section study the regulation of opioid peptides and tachykinins (such as substance P) at the cellular and molecular level, trying to elucidate the function(s) of these peptides in the nervous system. For this purpose, three separate, yet interrelated, research projects have been conducted: a) identification of the molecular mechanisms underlying the regulation of the opioid peptides and tachykinins by the dopaminergic (DA) system in the basal ganglia: Recent results have clearly demonstrated that the DA system exerts an important regulation on the gene expression of enkephalin, dynorphin,and tachykinins, the three most abundant neuropeptides in the basal ganglia. Long-term blockade of DA transmission increases the biosynthesis of enkephalin, but decreases that of dynorphin and tachykinins. In contrast, long-term enhancement of DA transmission produces the opposite effect, suggesting that gene expression may be an important site of action for certain psychoactive drugs. Nuclear run-off and in situ hybridization techniques are being set up to study the regulation of gene expression at transcriptional levels; b) Evaluation of the interactions between neurotransmitters and neuropeptides using the adrenal gland as a model to study the molecular mechanisms underlying the regulation of gene expression of enkephalin and catecholamines, which are co-stored in adrenal chromaffin cells. Evidence was obtained indicating that the biosynthesis of enkephalin, tyrosine hydroxylase, and phenylethanolamine-N-methyl-transferase are all subject to transsynaptic regulation. Using chromaffin cell cultures, the roles of primary messenger systems (cholinergic and peptidergic mechanisms) on the expression of enkephalin- and catecholamine-synthesizing enzymes have been clarified. Currently, the roles of various second messenger systems and several protooncogenes in the regulation of gene expression of the afore-mentioned peptide and enzymes are being studied; c) another research project evaluated the relationship between opioid peptides and epileptic seizures. Seizure activity results in altered release and biosynthesis of both enkephalin and dynorphin in the hippocampus, suggesting that changes in the activity of these opioid peptides may participate in the development of seizures, or mediation of postictal behaviors. These studies provide additional avenues for understanding the etiology of epilepsy, and for development of new and better pharmacological therapies.

Research in the Peptide Neurochemistry Group is focused on the search for and isolation of peptide hormones in mammalian tissues related to amphibian peptides, determination of the pharmacological and physiological activities of neuropeptides, and biochemical analysis of their action. Recent studies from this group reported that isolation and sequencing (using FAB-tandem mass spectrometry) of a mammalian peptide (PHLIP-8) which cross-reacts with antisera to physalaemin (PHY), an amphibian skin peptide structurally related to substance P. PHLIP-8, which contains sequence similarity to the N-terminal region of PHY, is a fragment of a large glycoprotein precursor. Current efforts concern the isolation of sufficient quantities of a new milk component for structure determination; this apparent peptide has potent bombesin (BOM)-like uterokinin activity. Additional studies on neuromedin B-like material (NB-LI) (a neuropeptide related to bombesin) using highly specific antisera against NB, revealed that its levels in rat pituitary, hypothalamus, and hippocampus undergo alterations during lactation and approach control values during weaning. Dermorphin is an opioid heptapeptide with a naturally occuring D-amino acid. Researchers in the Peptide Neurochemistry Group analyzed binding of dermorphin and numerous dimeric analogues to brain membrane receptor sites. (Dimeric peptides are two molecules covalently bound either directly through the C-termini or with intervening methylene units of various chain lengths.) Recent data revealed that these peptides bind μ-type receptors and provide a correlation that central μ receptors may be involved with peripheral bioactivity.

The Neurophysiology Group conducted research studies to understand basic mechanisms underlying modulation of neuronal function. The hippocampal formation was chosen as a "model" for these studies since it has been well defined anatomically, physiologically, and neurochemically, and it exhibits neuronal plasticity. The research focused on two major topics: a) modulation of neuronal function by neuropeptides (especially opioid peptides); and b) the role of zinc in the mossy fibers of the hippocampal formation. The studies utilized in vivo models for elicitation of seizure activity, the in vitro hippocampal slice technique, and primary cultures of rat hippocampal cells. The experimental approach was designed to investigate the biophysics and molecular biology of excitable cells, and the feedforward and feedback mechanisms influencing neuronal plasticity in the three major fiber tracts (perforant path, mossy fibers and Schaffer collaterals) within the hippocampal formation. The studies provided initial information on the conditions which affect release of the hippocampal endogenous opioids, the sites within the hippocampus and the cell types affected by their release and the mechanism(s) whereby they alter neuronal excitability within the hippocampus.

The overall goal of the Neurobehavioral Section is to understand the compensatory and modulatory processes associated with progressive degeneration and regeneration in the central nervous system, in the hope that this research may lead to a better understanding of certain neurodegenerative diseases, especially those associated with cognitive dysfunction, such as Senile Dementia of the Alzheimer's Type (SDAT).

Recent studies employed chemical probes to selectively destroy neuronal populations associated with cognitive function. Agents such as AF-64A (cholinergic neurotoxin), ibotenic acid (kills cell bodies, spares fibers), colchicine (kills hippocampal granule cells) or n-methyl-d-aspartate (activates n-MDA receptors) were infused into areas such as the hippocampus, septum, nucleus basalis and dose- and time-dependent effects on cognitive function were measured and associated with neurotransmitter and neuromodulatory changes and loss of target neuronal cells. One of the most promising models is colchicine, which binds to tubulin and inhibits synthesis of microtubules. When infused into the hippocampus, it interferes with working memory, the type known to be affected in SDAT. Other studies have focused on the capability of fetal hippocampal implants to promote recovery of function. The results indicate that the neurobehavioral alterations produced by direct administration of colchicine of nMDA into the hippocampus can be attentuated by implanted fetal hippocampal cells. These results suggest the possible use of hippocampal fetal implants in neurodegenerative diseases associated with cognitive dysfunction. Future experiments will examine ability of fetal implants to alter the progress of other neurological diseases, and will also examine the conditions under which viability and growth can be facilitated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70090-05 LMIN

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neuroendocrine and Neurochemical Regulation of Gonadal Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Negro-Vilar	Chief, LMIN	LMIN	NIEHS
OTHERS:	M.D. Culler	Senior Staff Fellow	LMIN	NIEHS
	W.C. Wetsel	Senior Staff Fellow	LMIN	NIEHS
	M.M. Valenca	Visiting Fellow	LMIN	NIEHS
	F. Lopez	Visiting Fellow	LMIN	NIEHS
	M. Ching	Exper	LMIN	NIEHS

COOPERATING UNITS *(if any)*

University of North Carolina, Department of Anatomy, Chapel Hill, NC; University of Pécs, Department of Anatomy, Pécs, Hungary.

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Reproductive Neuroendocrinology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	2.0	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recent efforts were directed to understand the cellular and subcellular mechanisms regulating the release of luteinizing hormone-releasing hormone (LHRH) and other hypothalamic peptides that participate in the modulation of pituitary hormone release. Specific studies were conducted to elucidate the role of gabaergic neurotransmitters in the release of LHRH from nerve terminals and the nature of the specific gabaergic receptors involved in the neuronal activation that precedes LHRH release. Additional studies evaluated other events that participate in the peptide-release process, i.e. the involvement of arachidonate metabolites in amplifying or modifying the response to key neurotransmitters, and the additional role played by intracellular messengers such as Ca^{+2}, protein kinase C, and other putative intracellular messengers derived from the metabolism of membrane phospholipids. Other parts of the project were directed to perform an in-depth analysis *in vivo* of the changes in LHRH prohormone levels and processing in discrete brain nuclei that are known to be involved in regulation of gonadal function. Different experimental paradigms were employed to recreate situations calling for an enhanced (or altered) function of the hypothalamic-pituitary-gonadal axis, such as steroid-feedback manipulations, pregnancy, lactation, estrous cycle, stress, ablation of selected endocrine glands or brain areas, etc. These studies utilized a combination of microdissection, radioimmunoassay, chromatographic and immunocytochemical approaches to better understand the changes in processing of peptide prohormone molecules in different brain regions as well as the release of different prohormone fragments from nerve terminals *in vitro*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70092-05 LMIN

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cellular and Molecular Mechanisms Mediating Peptide Hormone Action

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Negro-Vilar	Chief, LMIN	LMIN	NIEHS
OTHERS:	M.D. Culler	Senior Staff Fellow	LMIN	NIEHS
	W. Wetsel	Senior Staff Fellow	LMIN	NIEHS
	M. Ching	Expert	LMIN	NIEHS
	T. Iuone/M.M. Valenca	Visiting Fellow	LMIN	NIEHS
	F. Lopez/I. Wanderley	Guest Researcher	LMIN	NIEHS
	M. Conti	IPA	LMIN	NIEHS

COOPERATING UNITS *(if any)*

UNC, Department of Anatomy, Chapel Hill, NC; Yale University Medical School, Department of Obstetrics and Gynecology, New Haven, CT.

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Reproductive Neuroendocrinology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.3	2.0	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Evaluation of the cellular and molecular mechanisms mediating peptide hormone secretion constitutes an important component of our research efforts. The close interrelationships mediating neuroendocrine responses within the hypothalamic-pituitary-gonadal/adrenal system offer an excellent opportunity to analyze some unique characteristics of peptide-peptide, peptide-amine, and peptide-amine-steroid interactions. Studies using pituitary cell cultures were directed to evaluate the precise mechanisms through which different peptidergic or aminergic secretagogues enhance or suppress peptide hormone release. A main target of our research efforts has been to analyze the role of hormonal input signal in modifying cellular responses, using a computerized perifusion system that can exquisitely regulate the delivery of appropriately-designed hormone signals to cells or tissues (in vitro). Additional studies were conducted to evaluate characteristics of hormone-receptor interactions (post-receptor as well as transmembrane events involved in the hormone-release process) and to define the specific intracellular messengers transducing the action of key hypothalamic peptides involved in pituitary hormone release.

A major part of this project included work directed to understand the role of pulsatile hormone secretion in providing coded messages for the activation of endocrine target tissues. Several experiments were conducted to analyze the pulsatile secretion pattern of different peptide hormones (LHRH, LH, ACTH, Prolactin) that are intimately involved in the regulation of endocrine and reproductive functions. The characteristic pattern of secretion of each hormone was defined as well as the role of certain neural or gonadal factors in modulating the pulsatile pattern. Qualitative differences in pulsatile peptide secretion were uncovered. The information obtained provides a clearer understanding of the mechanisms regulating episodic hormone secretion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70096-04 LMIN

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Pulsatile Gonadotropin Secretion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael D. Culler	Senior Staff Fellow	LMIN	NIEHS
OTHERS:	Andres Negro-Vilar	Chief, LMIN	LMIN	NIEHS

COOPERATING UNITS *(if any)*

Dept. of Anatomy, University of North Carolina, Chapel Hill, NC; Dept. of Anatomy, University of Pécs Medical School, Pécs Hungary.

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Reproductive Neuroendocrinology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	0.9	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our previous studies in this project demonstrated that all aspects of LH secretion are dependent on the brain peptide, LHRH (GnRH), but that FSH secretion has two components: basal FSH secretion which is dependent on a LHRH sensitive component and pulsatile FSH secretion which is independent of LHRH and is probably regulated by a putative FSH-releasing hormone. Two candidates for these FSH-affecting components, recently discovered by recombinant DNA methodology, are a peptide that we have demonstrated previously to be a portion of the LHRH prohormone and a gonadal factor termed inhibin. We have produced antiserum against both of these new factors. In studies of the LHRH prohormone, we have demonstrated that the synthesis and processing of the LHRH prohormone is dependent on gonadal steroids. Studies to determine the significance of bioactivity associated with processed fragments of the prohormone are ongoing. Using the inhibin antiserum, we have localized inhibin in testicular Sertoli cells and ovarian granulosa cells. Ongoing studies with cultured rat Sertoli cells are revealing the mechanisms that regulate inhibin secretion. Using the antiserum to neutralize endogenous inhibin in vivo we demonstrated that inhibin affects only the basal aspects of FSH secretion. Neutralizing inhibin at different ages, we have demonstrated that endogenous inhibin actively suppresses FSH secretion prior to, but not after, puberty in the male and only after the initiation of puberty in the female. Restructuring of the inhibin subunits produces an FSH-releasing factor termed activin. We have very recently produced antiserum against activin and have begun studies to determine the physiology of this factor. The results from these studies have already redefined long standing concepts of the mechanisms regulating gonadotropin secretion and will continue to advance our understanding of these mechanisms in the future.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90033-06 LMIN

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Milk Bombesin and Kinins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	William E. Wilson	Research Chemist	LMIN	NIEHS
OTHERS:	L.H. Lazarus	Research Chemist	LMIN	NIEHS
	B.J. Irons	Biologist	LMIN	NIEHS
	A. Guglietta	Visiting Associate	LMIN	NIEHS

COOPERATING UNITS (if any)

University of Kyoto, Japan; University of Rome, Italy; University of North Carolina, Chapel Hill, NC.

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Peptide Neurochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Efforts to determine the nature of the uterokinins which were associated with partially purified bombesin recovered from bovine milk, have led to purification of two of the kinins, one of which has been identified as bradykinin. This finding led to an examination of the present state of understanding of bradykinin in the central nervous system. Present proof that bradykinin occurs in the brain derives from evidence utilizing a combination of chromatographic, RIA and radioreceptor assays. The variability in quantitative estimates of bradykinin levels in various regions of the brain are presumed to reflect, in part, characteristics of antibodies used in RIAs. It apppears that gonadal steroids modulate bradykinin-like immunoreactivity in the pituitary, however, there is disagreement as to the qualitative and localized nature of such modulation; this may indicate that multiple kinins are being recognized in the RIAs. It is anticipated that lessons learned in purfication of milk uterokinins will be applied to determine the precise nature of the kinins which occur in brain tissues, e.g., hypothalamus and pituitary.

Since arachidonate metabolites have been demonstrated to mediate bradykinin receptor interactions, a more precise definition of the qualitative nature of brain bradykinin(s) will complement other studies in this laboratory which implicate arachidonate metabolites in central mediation of gastric acid regulation by various opiate peptides and bombesin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90034-05 LMIN

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Rabbit Stomach Peptide (Physalaemin-Like Material [PHLIM]) in Mammalian Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William E. Wilson	Research Chemist	LMIN	NIEHS
OTHERS:	L.H. Lazarus	Research Chemist	LMIN	NIEHS
	B.J. Irons	Biologist	LMIN	NIEHS
	A. Guglietta	Visiting Associate	LMIN	NIEHS

COOPERATING UNITS *(if any)*

University of Kyoto, Japan; University of Rome, Italy; University of North Carolina, Chapel Hill, NC.

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Peptide Neurochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Physalaemin-like immunoreactive material (PHLIM) is a relatively prominent rabbit stomach macromolecule which has properties of mucin; it is assumed to possess the N-terminal amino acid sequence which was found in PHLIP-8 (<Glu-Val-Asp-Pro-Asn-Ile-Gln-Ala-OH). Our proposal to determine whether a high specific activity analog of physalaemin may exist in the central nervous system (perhaps, in precursor form as a component of a glycoprotein) has yet to be performed because of difficulties in obtaining a high titer antiserum.

An attempt will be made to obtain a mouse monoclonal antibody in order to determine whether distribution of a PHLIP-8 precursor in the central nervous system will provide immunohistochemical confirmation of prior observations obtained in this laboratory using polyclonal antisera to physalaemin. This investigation also has implications relative of oat cell carcinoma (which contains a physalaemin-related peptide) and to diseases involving glycoproteins in the mammalian digestive and respiratory tracts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90039-05 LMIN

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Modulation of Brain Opioids and Tachykinins by Neurotransmitter Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jau-Shyong Hong	Pharmacologist	LMIN	NIEHS
OTHERS:	H.K. Jiang	Guest Worker	LMIN	NIEHS
	P. Hudson	Biologist	LMIN	NIEHS
	M. Stachowiak	Senior Staff Fellow	LMIN	NIEHS
	H.A. Tilson	Pharmacologist	LMIN	NIEHS
	C. Hamm	Electronics Technician	LMIN	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Neuropharmacology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	0.3	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

  ☐ (a1) Minors

  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project were: 1) to understand the basic biosynthetic process of opioid peptide(enkephalin and dynorphin) and tachykinin (substance P) systems, 2) to elucidate how the classical neurotransmitter systems, such as dopamine (DA) interact with opioid peptides and tachykinins in the basal ganglia. Previous works have shown that blockade of dopaminergic transmission increases the biosynthesis of enkephalins, but decreases that of dynorphins and tachykinins in the basal ganglia. This study examined the effects of enhanced dopaminergic transmission on the expression of opioid peptide and tachykinin genes. For this purpose, apomorphine, a DA receptor agonist, and D-amphetamine, a DA releaser were employed. Repeated injections of either DA agonist increased the abundance of mRNA and the peptide content of substance P and dynorphin, but not enkephalin in the striatum. To determine whether the effects of repeated injections of DA agonists or antagonists on the peptides were a direct action on the nigrostriatal DA pathway or an indirect effect through other brain regions, a unilateral nigral 6-hydroxydopamine (6-OHDA) lesion model was used. Degeneration of nigrostriatal DA neurons prevented the D-amphetamine-induced increase in the levels of dynorphin and substance P in the basal ganglia. In contrast, the apomorphine-induced increase in the levels of these two peptides were greatly enhanced in the lesioned side as compared with the contralateral unlesioned side, presumably due to the supersensitivity of the DA receptors. It is interesting to note that the potentiation of apomorphine-induced increases in dynorphin and substance P only occurred in rats with more than 90% depletion of DA in the striatum. This phenomenon can be interpreted by our recent finding that the release of striatal DA measured by microdialysis procedure remained almost normal in rats with less than 90% depletion of striatal DA by 6-OHDA lesion. These results demonstrate the unusual great plasticity of the nigrostriatal pathway to maintain its transmission function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90042-03 LMIN

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Models of Neurodegenerative Processes Involving Cognitive and Motor Dysfunction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Hugh A. Tilson	Pharmacologist	LMIN	NIEHS
OTHERS:	R. McLamb	Biologist	LMIN	NIEHS
	K. Nanry	Psychologist	LMIN	NIEHS
	W. Mundy	Staff Fellow	LMIN	NIEHS
	B. Rogers	Biologist	LMIN	NIEHS
	C. Hamm	Electronics Engineer	LMIN	NIEHS
	R. Schwartz	Guest Worker	LMIN	NIEHS

COOPERATING UNITS *(if any)*

Duke University

LAB/BRANCH
Laboratory of Molecular and Integrative Neuroscience

SECTION
Neurobehavioral

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.59	1.42	3.17

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this research program is to study compensation and recovery of function that occurs following experimentally induced neurodegeneration in the central nervous system. Stereotaxic infusion of colchicine into the hippocampus of rats preferentially destroys dentate gyrus granule cells and mossy fibers and elicits reactive synaptogenesis from the cholinergic septohippocampal pathway. Intradentate colchicine causes specific changes in in vivo sensitivity to muscarinic cholinergic receptor agonists, increases in cholineacetyltransferase (ACHE) and cholineacetyltransferase (CHAT), two presynaptic markers for acetylcholine, and a decrease in the number, but not affinity, of muscarinic cholinergic receptors in the hippocampus. Agonist-mediated turnover of phospho-inositol was significantly increased in colchicine-treated animals suggesting hypersensitivity of the muscarinic receptor. Future studies will determine the specificity of this change in phosphoinositol turnover and attempt to examine possible colchicine-induced changes in the receptor-GTP coupling mechanism. The intrahippocampal colchicine model has been used to study the role of trophic factors in mediating recovery of function. Hyperactivity and deficits in working memory produced by intradentate colchicine were ameliorated by injection of a suspension of undifferentiated fetal cells into the hippocampus. Subsequent studies found that Timm-stained (for zinc) and enkephalin (ENK) immunoreactive (IR) fibers clustered around presumptive pyramidal cells 4 weeks after implant while ENK-IR and dynorphin-IR were present in grafted mossy fibers 6-12 weeks after implant. In situ hybridization histochemistry is being used to identify dentate granule cells in the grafts. Other experiments found that hippocampal fetal cell suspensions also ameliorated the cognitive deficits produced by N-methyl-D-aspartate. Future studies will characterize the conditions leading to the facilitative effects of the fetal implants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 90043-03 LMIN

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Zinc in Synaptic Transmission in the Hippocampal Formation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Clifford L. Mitchell	Pharmacologist	LMIN	NIEHS
OTHERS:	J.S. Hong	Pharmacologist	LMIN	NIEHS
	J. McGinty	Assoc. Professor	East Carolina	University

COOPERATING UNITS *(if any)*
Department of Anatomy, East Carolina University

LAB/BRANCH
Laboratory of Molecular and Integrative Neuroscience

SECTION
Neurophysiology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	0.5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several pieces of evidence suggest that endogenous opioids and zinc may interact to regulate neuronal excitability within the hippocampal formation. The purpose of this project is to conduct a systematic investigation into the effects of zinc on hippocampal neuronal excitability, with an emphasis on its interaction with enkephalin. The goal is to explain the nature of the effects of zinc and the mechanism(s) for its interaction with enkephalin. First it was necessary to determine the manner in which zinc levels were to be altered. As an initial approach we chose to attempt to alter zinc levels by systemic administration of zinc chloride or the intraviral zinc chelator, dithizone. The biological assay used was occurrence of wet dog shakes and seizures following subcutaneous administration of kainic acid (KA). We were unable to confirm the report of Porsche (IRCS Med. Sci. 11: 599, 1983) that subcutaneously administered Zn Cl_2 prevents KA induced seizures in rats. Instead, we found no effect of Zn Cl_2 in doses up to and including 100 mg/kg. This was true whether zinc was given before or after KA. In contrast, intraperitoneal injection of dithizone (12.5-100 mg/kg) or diethyldithiocarbamate (100-400 mg/kg) has a profound and dose related effect on the effects of KA. When given 15 minutes after the subcutaneous injection of KA, they markedly potentiate KA activity. They also produce a transient decrease in hippocampal levels of enkephalin and dynorphin. They also produce transient increases in the hippocampal levels of a number of amino acids (viz., taurine, glutamate, glutamine, and GABA). These effects are associated with reduced levels of hippocampal zinc (as measured by Timm staining of the hippocampus). It appears, then, that dithizone and diethyldithiocarbamate may prove to be useful tools for exploring the actions of zinc on the hippocampus. Work in progress involves: (1) further characterization of the changes in peptide and amino acids induced by these compounds, and (2) examination of their electrophysiological effects on the hippocampus.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90044-03 LMIN

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Modulation of Neuronal Function by Neuropeptides and Steroid Hormones

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Clifford L. Mitchell	Pharmacologist	LMIN	NIEHS
OTHERS:	J.S. Hong	Pharmacologist	LMIN	NIEHS
	C.W. Xie	Visiting Fellow	LMIN	NIEHS
	P. Lee	Visiting Fellow	LMIN	NIEHS
	J. McGinty	Assoc. Professor	East Carolina	University

COOPERATING UNITS *(if any)*

Department of Anatomy, East Carolina University

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Neurophysiology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	0.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Considerable work in this laboratory has focused on the role of enkephalin and dynorphin in seizure activity and related sezuelae. Among other things, this work has implicated enkephalin as playing a major role in the elucidation of a phenomenon in rats known as "wet dog shakes" (WDS). This work has also implicated the dentate granule cells (DGCs) as being necessary for the elicitation of WDS at least with respect to induction by kainic acid or by stimulation of the perforant path (PP). The first objective of this project was to develop a method of electrical stimulation of the PP which would elicit WDS consistently and repeatedly in the absence of an overt seizure. Using this method, we have demonstrated that stimulation of PP under conditions which elicit WDS produces a significant decrease in hippocampal levels of enkephalin and dynorphin. Levels of these substances are not altered by stimulus parameters insufficient to elicit WDS. Moreover, intraventricular injection of either an opioid mu receptor (β-FNA) or delta receptor (ICI174864) antagonist reduced the number of WDS elicited by PP stimulation. These data provide the first evidence that endogenous opioids are released by PP stimulation and lend further support to the notion that they play a role in regulation of hippocampal excitability. Current studies have demonstrated that naloxone, an opioid antagonist, reduces the number of WDS and tends to shorten the duration of electrical seizure activity induced by PP stimulation. The most striking effect of naloxone, however, is to markedly reduce the number of WDS at short (< 50 sec) durations of seizure activity. Lesion studies have demonstrated that a site involving the DGCs within the ventral hippocampus is critical for elicitation of WDS by PP stimulation. Studies in progress concern the nature of the differences between the dorsal and ventral hippocampus responsible for this phenomenon.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90045-03 LMIN

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on the Relationship between Opioid Peptides and Seizures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jau-Shyong Hong	Pharmacologist	LMIN	NIEHS
OTHERS:	P. Lee	Visiting Fellow	LMIN	NIEHS
	T. Xie	Visiting Fellow	LMIN	NIEHS
	C.L. Mitchell	Pharmacologist	LMIN	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Neuropharmacology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	0.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purposes of this project were: 1) to determine alterations in the metabolism of enkephalins and dynorphins in the limbic-basal ganglia regions after electroconvulsive shock (ECS) or after electrical kindling-induced seizures; 2) to study the possible roles of brain opioid peptides in seizure-induced changes in hippocampal excitability. Previous studies showed that both repeated ECS and electrical kindling to full behavioral convulsions produced striking differences in the hippocampal levels of certain opioid peptides: an increase in enkephalin level, but a drastic decrease in dynorphin level. This project was aimed to determine the underlying mechanism of these differential changes in peptide levels, by measuring the mRNA encoding these two peptides as an index of their biosynthesis. Repeated ECS (once per day for 6 days) or prepyriform cortical kindling to stage 5 convulsions produced a drastic reduction in the abundance of mRNA encoding dynorphin in the hippocampus, while an increase was observed in the hypothalamus and striatum. Thus the changes of mRNA paralleled the alterations of dynorphin levels in these brain regions. These data suggest that ECS- and kindling-induced seizures decrease the biosynthesis of dynorphin in the hippocampus. In contrast, the increase in the hippocampal level of enkephalin after ECS or kindling was not accompanied by an increased amount of mRNA encoding enkephalins. Instead, a large increase of enkephalin mRNA was found in the entorhinal cortex, where the cell bodies of enkephalin-containing neurons projecting to the hippocampus are located. These data suggest that ECS- or kindling-induced increases in the level of hippocampal enkephalin are mainly derived from the entorhinal cortex through the perforant pathway. Since enkephalins and dynorphins have been shown to be potent in modulating hippocampal excitability, the differential regulation of these two opioid peptides may play important roles in mediating the postictal behaviors.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 90047-02 LMIN

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neuroregulatory Aspects of Neuromedin B

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lawrence H. Lazarus	Research Chemist	LMIN	NIEHS
OTHERS:	William E. Wilson	Research Chemist	LMIN	NIEHS
	B.J. Irons	Biologist	LMIN	NIEHS
	A. Guglietta	Visiting Associate	LMIN	NIEHS

COOPERATING UNITS *(if any)*
University of North Carolina, Chapel Hill, NC; University of Kyoto, Japan; Karolinska Institutet, Stockholm, Sweden; University of Torino, Italy.

LAB/BRANCH
Laboratory of Molecular and Integrative Neuroscience

SECTION
Peptide Neurochemistry

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was discontinued after the beginning of FY-88. Two articles were published on this topic: J. Neurosci. Meth. 23:161-172 (1988) and Ann. N.Y. Acad. Sci., in press (1988). Other manuscripts are being prepared by collaborators in Sweden and Italy dealing with the immunohistochemical distribution of neuromedin B in selected regions of mammalian and urodele brains using antisera to neuromedin B supplied by this Group. Using this techinique, we were unable to find an extensive neuromedin B-specific network of nerve fibers or cell bodies in rat brain. These data contrast to the broad distribution of bombesin-containing nerve fiber network: bombesin and neuromedin B belong to the same peptide family and contain 70% sequence similarity, but are derived from seperate genes. This lack of immunohistochemical evidence may be related to the specificity of the antisera or the relatively low concentration of neuromedin B in the tissue slices, or both. Further studies on its localization in the pituitary continues at the University of North Carolina.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 90048-03 LMIN

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Physiology and Pharmacology of Neuropeptides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lawrence H. Lazarus	Research Chemist	LMIN	NIEHS
OTHERS:	William E. Wilson	Research Chemist	LMIN	NIEHS
	B.J. Irons	Biologist	LMIN	NIEHS
	A. Guglietta	Visiting Associate	LMIN	NIEHS

COOPERATING UNITS *(if any)*
University of Rome, Italy; University of North Carolina, Chapel Hill, NC; Farmitalia Carlo Erba, Milan, Italy

LAB/BRANCH
Laboratory of Molecular and Integrative Neuroscience

SECTION
Peptide Neurochemistry

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The neuroregulation of peripheral physiological functions by several neuropeptides and LiCl continued with 1) a comparative analysis between central and peripheral mode of action, 2) involvement of inhibitors of arachidonate metabolism in brain, and 3) correlation of brain μ-receptors to the inhibition of the gastric secretory processes. Dose response curves and time course analysis reveal that neuropeptides act only within the brain (icv administration) to suppress gastric acid secretion. Salmon calcitonin, however, is the exception, being two times more effective sc than icv. Currently CGRP (calcitonin gene-related peptide), a peptide whose biological properties differ from that of calcitonin, is being investigated as well as CRF to determine the extent of involvement of ACTH/β-endorphin in the inhibition of gastric secretion. In addition, the mechanism of action of TRF and ANF, which both increase secretion, are under study, as positive controls. LiCl, a modulator of manic depression which reduces gastric ulcer formation in those patients, inhibits gastric acid release: icv>iv>sc administration in effectiveness. The direct involvement of neuropeptides and LiCl on preventing experimentally-induced ulcers is also being studied. In addition to the involvement of the cyclooxygenase pathway (through use of inhibitors which reverse the suppression of gastric secretion by neuropeptides), the lipooxygenase pathyway (which yields leukotrienes) is implicated, since the inhibitor NGA increases secretion in the presence of neuropeptides. The use of specific μ-receptor agonists were used to probe gastric secretion in rat brain symaptosomes: DAGO, a μ-specific enkephalin analogue, as well as dermorphin (DM) and dimeric DM analogues, suppress gastric secretion, while DADLE (a δ-agonist) is ineffective at the same dose. A positive correlation exists between the suppression of gastric acid output and high affinity μ-receptors. This research will terminate on 30 September 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90049-02 LMIN

PERIOD COVERED

October 1, 1988 to September 30; 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Regulation of the Hormonal Output from Adrenomedullary Chromaffin Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michal K. Stachowiak	Senior Staff Fellow	LMIN	NIEHS
OTHERS:	J.S. Hong	Pharmacologist	LMIN	NIEHS
	A. Poisner	IPA	LMIN	NIEHS
	V. Owyang	Biologist	LMIN	NIEHS
	P. Hudson	Biologist	LMIN	NIEHS
	S. An	SIS	LMIN	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Neuropharmacology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In this project we study the mechanisms underlying coordinate and selective regulation of the hormonal output from the adrenal medulla (AM). Enhanced catecholamine and enkephalin release is accompanied with changes in mRNA levels of proenkephalin A (pEK) and catecholamine biosynthetic enzymes: tyrosine hydroxylase (TH) and phenylethanolamine N-methyltransferase (PNMT). The goal of this project was to examine: 1) nature of the extracellular and intracellular signals involved in the control of TH, PNMT and pEK mRNA levels; 2) role of transcription and post-transcriptional mechanisms in the processes; 3) whether expression of TH, PNMT, and pEK genes could be differentially regulated; 4) cis-and trans-acting factors involved in these regulations. We have used the following experimental models: 1) electrical stimulation of splanchnic nerves; 2) activation of the neural input by hypoglycemia and cold stress; 3) hypophysectomy and administration of glucocorticoids; 4) primary cultures of bovine AM cells. TH, PNMT and pEK mRNA levels were differentially regulated by neural inputs and by direct action of hormones (glucocorticoids and angiotensin) on the adrenal medullary cells. Inductions by depolarization and angiotensin were mediated by: influx Ca^{2+}, calmodulin, and kinase C. Stimulation of adenylate cyclase, and depletion of the intracellular catecholamines were also associated with the transcriptionally mediated changes in mRNA levels. Currently we examine roles of Ca^{2+}, kinase C, and muscarinic receptors in the regulation of TH, PNMT, and pEK genes. We also determine nature of signals mediating multilevel actions of angiotensin on AM cells demonstrated in this project: short-term regulation of catecholamines and enkephalin release, activation of TH, and the long-term regulation of gene expression. Our studies may contribute to a better understanding of mechanisms of information processing in multiple transmitter cells and the regulation of transmitter phenotype in the sympatho-adrenal system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90050-02 LMIN

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Roles of Opioid Peptides in the Regulation of Hippocampal Excitability

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jau-Shyong Hong	Pharmacologist	LMIN	NIEHS
OTHERS:	P. Lee	Visiting Fellow	LMIN	NIEHS
	P.M. Hudson	Biologist	LMIN	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Neuropharmacology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.25	1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Roles of opioid peptides in the regulation of hippocampal excitability are under intensive study after the discovery of endogenous opiates in the brain. Intraventricular administration of opioid peptides elicited epileptiform discharges and wet dog shakes (WDS) in rats. We have shown that injection of specific mu receptor agonist [NMe-Phe^3-D-Pro^4]-morphiceptin (PL017) into the ventral hippocampus produced behavioral convulsions and WDS in rats. The purpose of this study was to further investigate the role of other hippocampal opioid receptor subtypes, delta and kappa, on the expression of convulsions and WDS. Selective mu agonist, PL017 or [D-Ala^2-N-methyl-Phe^3-Gly^5-ol]-enkephalin, produced convulsions and WDS when unilaterally injected into the ventral hippocampus. The mixed mu-delta agonist [D-Ala^2,D-Leu^5]-enkephalin (DADLE) also elicited such behavioral changes, but its effect was less potent than the mu agonists. Similar treatment with the selective delta agonist, [D-Pen^2,^{5}]enkephalin or the selective kappa agonists, U-50488H, dynorphin-A amide or dynorphin A(1-8), did not produce convulsions or WDS. DADLE-induced convulsions and WDS were antagonized by mu receptor antagonist, β-funaltrexamine, but not by selective delta receptor antagonist, ICI-174864 . These results demonstrate that opioid-induced convulsions and WDS in rats are mediated exclusively by mu but not delta or kappa opioid receptors in the ventral hippocampus. The injection of a high dose of PL017 into the dorsal hippocampus or other brain regions such as frontal cortex, striatum and amygdala did not produce convulsions or WDS. These findings suggest that the ventral hippocampus is an important site for the expression of opioid-induced convulsions and WDS. Further studies are planned, by using hippocampal slice as a tool, to determine the response of dorsal against ventral hippocampus on opioid peptides induced excitation. In vitro release of GABA will be determined to test the hypothesis that opiates induced excitability in hippocampus may be mediated through the inhibition of GABA release.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90051-02 LMIN

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Brainstem and Spinal Cord Modulation of Neurological Motor Dysfunction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Hugh A. Tilson	Pharmacologist	LMIN	NIEHS
OTHERS:	D. Herr	Biologist	LMIN	NIEHS
	C. Hamm	Electronics Engineer	LMIN	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Neurobehavioral

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.58	0.08	0.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this research program is to develop in rats models of neurodegeneration using neurotoxicants to mimic features of neurological disorders, such as Alzheimer's Disease (AD). Microinfusion of colchicine, a neurotoxicant that binds to tubulin and disrupts assembly of microtubules, into the hippocampus, an area known to degenerate in AD, destroys dentate gyrus granule cells and mossy fibers preferentially and disrupts the acquisition and retention of working and reference memory only if it is performed in a spatial context. Working or short-term memory is affected early in AD, while reference or long-term memory is affected late in AD. These data indicate the importance of the hippocampus for memory partricularly if performed in a spatial context. Infusion of colchicine into the nucleus basalis, an area containing cell bodies for cholinergic neurons that project to the cortex and known to degenerate in AD, disrupted the acquisition and retention of spatial, working memory; reference memory was not affected. Further characterization of the colchicine effect revealed a relatively localized destruction of cells in the nucleus basalis which was associated with a regionally specific decrease in cholineacetyltransferase activity in the cortex. Receptor binding studies found a decrease in presumably presynaptic nicotinic receptors, while post-synaptic muscarinic receptors were not affected. Recent studies have shown that overexcitation of receptors for excitatory amino acid neurotransmitters can result in neurotoxicity and has been suggested as a possible etiological factor in AD. Microinfusion of N-MDA into the hippocampus produced dose-dependent loss of neurons and deficits in a spatial reference memory task. These effects could be blocked by pretreatment with MK-801, a noncompetitive receptor blocker for N-MDA receptors. These results indicate the potential usefulness of agents such as MK-801 as possible therapeutic agents for neurodegenerative disorders.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90052-01 LMIN

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Compensation and Recovery of Function in the Central Nervous System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Hugh A. Tilson	Pharmacologist	LMIN	NIEHS
OTHERS:	C. Hamm	Electronics Engineer	LMIN	NIEHS
	W. Zhang & P. Tandon	Visiting Fellows	LMIN	NIEHS
	K. Nanry	Psychologist	LMIN	NIEHS
	J. Peterson	Biologist	LMIN	NIEHS
	P. Pediaditakis	Biological Aid	LMIN	NIEHS
Y. Wilsdon, J. McGinty, G. Harry		Guest Worker	LMIN	NIEHS

COOPERATING UNITS *(if any)*

East Carolina University; University of North Carolina (Psychology Department and Biological Sciences Research Center)

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Neurobehavioral

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.67	2.5	2.17

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this research is to study the role of the brain stem and spinal cord in motor dysfunction, such as tremor and hyperreflexia, which are signs frequently associated with neurological disorders, such as Parkinson's and Alzheimer's Disease. These investigations used p,p'-DDT, which interferes with Na^+ channels causing repetitive firing of motor neurons. A potential facilitative role of spinal α_1 receptors was hypothesized and examined by intrathecally infusing prazosin, an α_1 receptor blocker, and in vivo binding techniques. The time and dose-dependent tremor produced by p,p'-DDT has been shown previously to be blocked by phenytonin, which acts on Na^+ channels, and mephenesin, which blocks polysynaptic transmission in the spinal cord. Intrathecal administration of prazosin tended to reduce tremor produced by p,p'-DDT, but did so at doses that also reduced ambient levels of motor movement. Larger doses of prazosin significantly attenuated DDT-induced tremor and myoclonia and blocked both spinal and cortical α_1 receptors, suggesting involvement of supraspinal, facilitative α_1 -mediated effects. These experiments support the hypotheses that the in vivo signs of tremor and myoclonus are related to DDT's actions at Na^+ channels and involve polysynaptic transmission. Norepinephrine, acting at α_1 receptors, may facilitate the intensity of the neurotoxic signs. This effect may be mediated by α_1 receptors in several regions of the neuroaxis, rather than solely at the level of the spinal cord. This is the last report for this project; no further work is planned.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90053-01 LMIN

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropeptides: Molecular Mechansim of Action

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Lawrence H. Lazarus	Research Chemist	LMIN	NIEHS
OTHERS:	William E. Wilson	Research Chemist	LMIN	NIEHS
	B.J. Irons	Biologist	LMIN	NIEHS
	A. Guglietta	Visiting Associate	LMIN	NIEHS

COOPERATING UNITS (if any)

University of Turin, Italy; University of North Carolina, Chapel Hill, NC; Farmitalia Carlo Erba, Milan, Italy.

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Peptide Neurochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Dermorphin (DM), an amphibian opioid heptapeptide which contains a natural D-Ala in position 2, N-terminal DM analogues and a series of dimeric DM analogues (coupled through the C-terminus) were analyzed for μ- and δ-receptor affinities on rat brain synaptosomes. Binding assays used ^{3}H-DAGO for μ-receptors and ^{3}HDADLE for δ-receptors (carried out in the presence of [N-Me-Phe^3,D-Pro^4] morphiceptin to suppress interaction with μ-receptors). Dimeric DM tetrapeptides weakly interacted with μ-receptors; in particular, the analogue with a 12 methylene unit bridge between peptide chains had poor μ- and δ-affinities and no selectivity. However, analogue with an ethylene bridge containing D-Arg^2 and Sar^4 had high μ-affinity, μ-selectivity, and correlated to the inhibition of gastric acid secretion. All the dimeric DM pentapeptides had very high μ-affinities, with moderate to low μ-selectivity, but correlated in a positive manner to the suppression of gastric secretion. Whereas D-Arg^2 and Sar^4 increased μ-affinity and selectivity in the dimeric tetrapeptides, they decreased these parameters in the pentapeptides. Dimeric DM and DM exhibited high affinity for μ-receptors and selectivity approaching DAGO and had a positive correlation to the inhibitory effect in the gastric secretory process. As a control for the binding specificity, the [L-Ala^2]DM analogue was tested: it bound to μ-receptors with an affinity 3-orders of magnitude less than DM, which is in keeping with its minimal bioactivity. Thus, these studies provide evidence that high affinity μ-receptors in brain tissue correlate in a positive manner to the inhibition of gastric acid output in stomach by the central (icv) administration of DM and selected dimeric DM analogues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90054-01 LMIN

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Biosynthesis, Processing and Secretion of Neuropeptides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William C. Wetsel	Senior Staff Fellow	LMIN	NIEHS
OTHERS:	Andrés Negro-Vilar	Chief, LMIN	LMIN	NIEHS
	Gail Wisniewski	Biologist	LMIN	NIEHS
	Bill Scarlett	BLT	LMIN	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Reproductive Neuroendocrinology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	0.9	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A question which is central to neurobiology relates to the regulation of biosynthesis, processing and secretion of peptides. We have examined these cellular processes using the LHRH prohormone as a model. Both nerve terminals (containing processed products) and cell bodies with their axons (containing proLHRH and partially processed products) were dissected separately. Materials from these tissues were separated by size using HPLC. Fractions were screened with antisera which were specific for different regions of the proLHRH molecule. The proLHRH was cleaved into 2 major products: LHRH and GAP_{1-56}. While LHRH was not metabolized further, GAP was cleaved into 3500 and 2800 molecular weight materials which may represent GAP_{1-32} and GAP_{34-56}. In order to study regulation of biosynthesis and processing, we compared brain levels of all proLHRH-derived peptides from castrate and intact rats. Castration reduced levels of proLHRH and all its products relative to intacts; the pathway of processing was unaffected. In additional studies, nerve terminal fragments from brains of rats castrated for 14-days released approximately 2-fold less LHRH and GAP_{1-56} into the media than intact rats. Since tissue stores of both peptides in castrates were also reduced by 2-fold, the data were expressed in terms of the precent of peptide released. Similar percentages of peptides were released from intact and castrated rats under basal and [K^+]-stimulated conditions. By contrast, castrates secreted a smaller percentage of both peptides in response to phorbol ester stimulation than intacts. Our results are significant because they describe the biosynthesis and metabolism of the LHRH precursor. In addition, our findings show that biosynthesis and secretion of proLHRH peptides may be regulated by testicular factors. Future studies will evaluate in greater detail the regulation of biosynthesis and processing, the nature of the proLHRH products, and the mechanism(s) responsible for the defect in the protein kinase C pathway in castrated rats.

LABORATORY OF PULMONARY PATHOBIOLOGY

LABORATORY OF PULMONARY PATHOBIOLOGY
Summary statement

The respiratory tract is an organ system which is exposed to a multitude of environmental insults. It is equipped with a number of cellular and biochemical defense systems protecting it from exogenous agents. Among these are the so called muco-ciliary clearance mechanism and the alveolar macrophage system, which are able to remove chemical and microbial agents from the lung, and the biochemical anti-oxidant defenses which protect the lung against oxygen free radicals. When these and other defense mechanisms fail, severe damage to the pulmonary tissues occurs, particularly when the insults are repetitious, and can result in chronic or even progressive disease such as emphysema, pulmonary fibrosis and lung cancer.

To design strategies for disease prevention and effective approaches to intervention in disease development, it is necessary to study the mechanisms of toxicity of various chemicals and it is essential to acquire a detailed understanding of the biology of this organ system at the cellular, biochemical and molecular level. We need to develop a thorough understanding of the mechanisms which govern growth and differentiation of various cell compartments, which regulate the interaction between different cell types such as fibroblasts and epithelial cells and which modulate the biochemical activity of such cells as secretory cells, vascular endothelium and macrophages to name but a few. Therefore this Laboratory is engaged in the study of normal cell biology of respiratory tract tissues as well as in the study of pathogenetic mechanisms of respiratory tract diseases.

The Laboratory of Pulmonary Pathobiology is divided into 4 sections. The Section of Pulmonary Cell Biology investigates the regulation of tracheo-bronchial epithelium at the cellular and molecular level. The Section Epithelial Carcinogenesis investigates mechanisms involved in neoplastic transformation of airway epithelium. The Section Biochemical Pathology studies secretions of the distal airways and the alveoli. The Section Pulmonary Pathology studies the pathogenesis of pulmonary fibrosis caused by asbestos and other toxic particles. In the following, two broad areas of investigation will be briefly discussed, as examples of the Laboratories current research activities.

Studies of the differentiation of tracheo-bronchial epithelium. The normal muco-ciliary epithelium of the conducting airways is composed of three major cell types, namely basal cells, secretory (mucous) cells and ciliated cells. However this pattern of differentiation can change rapidly when the epithelium is injured by various insults including toxic or carcinogenic air contaminants. The epithelial lining of the bronchi then changes to a squamous (epidermoid) phenotype. Similar changes are also known to occur during severe forms of vitamin A (retinoid) deficiency. The Laboratory is engaged in several studies concerned with normal and abnormal differentiation, one of which is aimed at identifying the stem cells of tracheo-bronchial epithelium. To establish the identity of stem cells in a tissue is important for several reasons: a) because many factors regulating growth and differentiation are presumed to act at the stem cell level and b) because carcinogens are believed to act by transforming stem cells. The hypothesis, which is currently heavily disputed, is, that the basal cells are the stem cells of the tracheo-bronchial epithelium. Tracheal basal cells were isolated by centrifugal elutriation, single cell derived basal cell clones were generated in culture and these clones were then transferred to an *in vivo* culture system, which fully supports expression of the muco-ciliary phenotype. These studies showed that basal cells are able to generate more basal cells and to give rise to mucous and ciliated cells, thereby fulfilling the criteria for stem cells of the tracheo-bronchial epithelium. Experiments are being designed to examine the regulation of basal cell differentiation by various regulatory peptides.

A related series of investigations is concerned with biochemical and molecular mechanisms of squamous cell differentiation, using cultures of tracheal or bronchial epithelial cells from various species including humans. Squamous differentiation was monitored with several biochemical markers (epidermal transglutaminase, cholesterol sulfate, specific cyto-keratins) and was found to proceed in at least two distinct steps: the first critical step is the commitment to terminal cell division (irreversible growth arrest) which can be triggered by addition or subtraction of the growth regulatory peptides TGFbeta and EGF, respectively. The second step is the expression of the squamous phenotype. This step was found to be regulatable by retinoids and calcium, retinoids inhibiting and calcium enhancing expression. Presently studies are underway to explore mechanisms of gene regulation during squamous differentiation. cDNA clones have been isolated which hybridize with mRNAs abundant in differentiated cells. These clones are used to study regulation of relevant genes by retinoids and TGFbeta. In the same context the role of the recently discovered nuclear retinoid receptors in the control of squamous differentiation is being investigated.

Studies on mechanisms of injury to the lung by toxic particles and fibers. Inhalation of many types of particles and fibers, e.g. silica particles and asbestos fibers, can cause damage to respiratory tract tissues and result in severe, progressive pulmonary fibrosis/pneumoconiosis. While considerable progress has been made in recent years, to elucidate mechanisms involved in the development of these crippling lung diseases, many critical steps involved in their pathogenesis are still poorly understood.

Researchers of this and other Laboratories showed, that upon deposition of toxic particles and fibers in airways several important pulmonary cell populations, notably type II alveolar cells and interstitial fibroblasts are stimulated in their biosynthetic activity (e.g. overproduction of pulmonary surfactant and of extracellular matrix) as well as in their proliferative activity. The mechanisms triggering these responses are by no means clear. The central hypothesis which is being examined by researchers of LPP is, that pulmonary macrophages, which were shown to be attracted to the site of particle deposition by a complement mediated mechanism, secret factors which stimulate the proliferation of pulmonary fibroblasts. (Similar mechanisms may also be involved in the stimulation of type II alveolar cells which were shown to proliferate in silica exposed lungs to become hypertrophic and to secrete alveolar surfactant in great excess). It was shown that alveolar as well intravascular macrophages produce a broad spectrum of arachidonic acid metabolites among which are several well characterized inflammatory mediators. The production of these metabolites was significantly enhanced by a variety of particles and fibers. Alveolar macrophages were further shown to secrete a number of different growth factors, including a homologue of platelet-derived growth factor (PDGF), a known fibroblast mitogen. Secretion of PDGF was enhanced by particle exposure. Subsequently it was demonstrated, that early passage rat lung fibroblasts exhibit receptors for the macrophage secreted PDGF-homologue. All of these studies support the notion that particle induced pulmonary fibrosis is mediated by macrophage derived factors. However, most of these studies were carried out in *in vitro* systems. Therefore it still remains to be proven that the factors produced by particle exposed macrophages cause fibroblast proliferation in vivo. Studies to examine this question are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES25020-06 LPP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of the Pulmonary Surfactant System and its Modification by Toxic Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G. E. R. Hook	Research Chemist	LPP, NIEHS
Others:	L. B. Gilmore	Biologist	LPP, NIEHS
	B. E. Miller	Graduate Student	LPP, NIEHS
	G. J. Baker	Visiting Fellow	LPP, NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Pulmonary Pathobiology

SECTION

Biochemical Pathology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.00	2.75	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Pulmonary surfactant is a complex mixture of lipids and proteins that prevents collapse of the alveoli and distal airways at low lung volumes. Silica dust causes massive increases in the surfactant content of the lungs but the mechanisms through which this occurs are not known. Examination of the rates at which surfactant phospholipids are synthesized, secreted, and cleared from the lungs of silica-treated rats revealed that expansion of the intra and extracellular pools of surfactant could be accounted for by imbalances between the rates at which surfactant phospholipids are synthesized by alveolar Type II cells, secreted, and then cleared from the alveoli. Under normal circumstances these rates of synthesis, secretion, and clearance are the same and the intra- and extra-cellular pools of surfactant do not change in size. Our studies indicate that central to the expansion of surfactant pools are alterations in the biosynthetic rates at which surfactant phospholipids are synthesized and that these increased biosynthetic rates are associated primarily with a subpopulation of Type II cells that are highly activated with regard to their ability to synthesize surfactant phospholipids. We have developed a method for the isolation of these activated Type II cells from the lungs of silica-treated rats and for the separation of those activated cells from the non-activated or normal Type II cells. Examination of the pathway by which phosphatidylcholine is synthesized reveals that increased synthesis is achieved by marked elevation in the activities of two enzymes; cytidylyltransferase and choline phosphotransferase. Activation of the surfactant pathway by silica may be mediated through these two critical enzymes in the biosynthetic pathway of phosphatidylcholine. In addition, it appears likely that these activated Type II cells play a role in the repair processes within the alveoli of the lungs. Preliminary evidence indicates that hyperactive Type II cells are also the proliferative cells within the alveoli. Future studies will involve further characterization of the hyperactive Type II cells and investigation of their role in repair processes within the lungs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 25021-05 LPP

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Differentiation of Tracheobronchial Epithelial Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.M. Jetten	Senior Staff Fellow	LPP, NIEHS
Others:	E.E. Floyd	Staff Fellow	LPP, NIEHS
	C. Nervi	Visting Fellow	LPP, NIEHS
	T. Vollberg	Staff Fellow	LPP, NIEHS
	M. George	Chemist	LPP, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Pulmonary Pathobiology

SECTION
Cell Biology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	4	1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies using in vitro cell culture systems of tracheobronchial epithelial cells provide useful models in which to study the regulation of proliferation and differentiation. The clonogenic cells derived from the tracheobronchial epithelium appears pluripotent. They have self-renewal capacity and can differentiate along a normal, muco-secretory pathway or a squamous cell pathway. Squamous differentiation in tracheobronchial epithelial cells is a multi-step process consisting of at least three stages. In the initial stage cells become committed to terminal cell division in which cells become irreversibly growth arrested. This is followed by the expression of the squamous differentiated phenotype and finally cornification. During squamous differentiation cells undergo changes in keratin expression. Some of these changes appear to be regulated at the post-transcriptional and likely at the translational or post-translational levels. Various factors such as several growth factors (TGFβ, EGF), calcium ions, retinoids and phorbol esters regulate the program of differentiation at different stages. Retinoids do not affect the commitment to terminal cell division but inhibit the expression of the differentiated phenotype. This inhibition occurs at very low concentrations indicating that high affinity sites are involved in this action. Bryostatins, polycyclic lactones produced by bryozoan Bugula neritina, can bind to and activate protein kinase C similar to phorbol esters. However, bryostatins do not induce terminal differentiation in tracheobronchial cells as phorbol esters do. Moreover, bryostatins inhibit the commitment to terminal differentiation and the expression of the differentiated phenotype induced by phorbol esters. Bryostatin may act via a target other than protein kinase C. Tracheobronchial epithelial cell lines have been established using SV40-large T antigen after infection of normal tracheobronchial cells with a retroviral shuttle vector. These cell lines and carcinoma lines isolated from lung tumors exhibit altered responses to growth and differentiation regulatory factors. Alterations in the commitment to differentiation must be a crucial step in the transition towards a tumor cell.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES-25023-05 LPP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cellular and Molecular Mechanisms of Progression of Transformed RTE Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Paul Nettesheim	Chief	LPP, NIEHS
Others:	C. Walker	Senior Staff Fellow	LPP, NIEHS
	P. Ferriola	Staff Fellow	LPP, NIEHS
	Z. Duniec	Visiting Fellow	LPP, NIEHS
	Y. Inayama	Visiting Associate	LPP, NIEHS
	S. Endo	Visiting Fellow	LPP, NIEHS
	T. Gray	Biologist	LPP, NIEHS
	M. Joyce	Technician	LPP, NIEHS

COOPERATING UNITS *(if any)*

Cell Biology Group
Biochemical Pathology Group

LAB/BRANCH

Laboratory of Pulmonary Pathobiology

SECTION

Epithelial Carcinogenesis Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8	5	3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The research efforts in the Epithelial Carcinogenesis Group are concerned with two major topics: 1) the regulation of growth and differentiation in tracheo-bronchial epithelium and 2) the mechanisms involved in multi-step neoplastic transformation of the same epithelium. A controversy has developed in recent years regarding the identity of the stem cells in the tracheo-bronchial epithelium. The old hypothesis stated that basal cells give rise to secretory and ciliated cells and act as primitive stem cells. This hypothesis has recently been challenged, because it was found, that following injury, the main proliferative cell compartment is the compartment of secretory cells.

To investigate this important problem, we isolated two different cell types from the airways of rabbits, using centrifugal elutriation procedures, namely bronchiolar Clara cells and tracheal basal cells. The highly purified cell preparations were then cultured in an in vivo culture system which fully supports the differentiation of all major tracheo-bronchial cell types. The results showed that Clara cells have stem cell properties, since they generate Clara cells as well as ciliated cells (but not basal cells or mucous cells). Tracheal basal cells grown under the same conditions generated basal cells, mucous cells and ciliated cells (but not Clara cells). These results suggest that Clara cells are the epithelial stem cells of the bronchiolar epithelium and that basal cells are the stem cells of the large conducting airways (trachea and bronchi); they further suggest that Clara cells and basal cells represent two separate stem lines of epithelial cells. Studies are now underway to define the various cell types in the airways more precisely with the use of biochemical and immunological cell surface markers. We are also examining the sensitivity of Clara and basal cells to factors such as retinoids, TGFbeta, Ca^{2+} and TPA which regulate and modulate epithelial cell differentiation. Another important ongoing project is to study the susceptibility of basal cells to chemical transforming agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES-25027-05 LPP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Identification and Characterization of Materials Secreted by Pulmonary Clara Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. E. R. Hook	Research Chemist	LPP, NIEHS
Others:	L. Gilmore	Biologist	LPP, NIEHS

COOPERATING UNITS *(if any)*

Cell Biology Group (A. M. Jetten)
Epithelial Carcinogenesis Group (P. Nettesheim)

LAB/BRANCH

Laboratory of Pulmonary Pathobiology

SECTION

Biochemical Pathology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The functions of the bronchiolar Clara cell are not known although it is generally believed that the cell is secretory. Using a model system, developed in this laboratory, we have identified a low molecular weight protein (Mr 12,500) as the major protein secreted by Clara cells. We have also identified the major secretory protein in pulmonary lavage effluents from the lungs of rabbits and developed a simple procedure for its isolation. Antiserum developed in goats against highly purified Clara cells (purity greater than 90%) has been used to localize Clara cell secretory proteins (CCSP) within the osmiophilic cytoplasmic granules of bronchiolar Clara cells indicating the storage nature of the granules and that secretion of the low molecular weight protein should occur primarily through a regulated pathway. However, we have been unable to demonstrate the regulated pathway and we now conclude that most secretion occurs via a constitutive pathway not involving the secretory granules at all. Using antiserum to CCSP we have demonstrated the presence of an immunogenically similar protein in uterine secretions from pseudopregnant rabbits. We have also isolated uteroglobin from uterine secretions, and with antibodies demonstrated that CCSP and uteroglobin are immunochemically similar proteins. These preliminary data indicate that the major secretory protein of the bronchiolar Clara cells of the rabbit may be uteroglobin. Future studies will seek to establish the realtionship of CCSP to uteroglobin and focus on the extracellular function of these low molecular weight secretory proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES-25030-02 LPP

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cellular and Biochemical Mechanisms of Particle-Induced Lung Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Arnold R. Brody	Research Biologist	LPP, NIEHS
Others:	R. Bennett	Staff Fellow	LPP, NIEHS
	R. Kumar	Fogarty Fellow	LPP, NIEHS
	T. Bertram	IPA	LPP, NIEHS
	J. Bonner	IRTA Fellow	LPP, NIEHS
	L. Bartling	Biologist	LPP, NIEHS
	A. Badgett	Biologist	LPP, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Pulmonary of Pathobiology

SECTION
Pulmonary Pathology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7	5	2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Research in the pulmonary pathology laboratory has been focused upon the basic biological mechanisms through which inhaled particles cause lung disease. We have developed models of asbestosis and silicosis using rats and mice and have shown that the disease process is initiated at junctions of bronchioles and alveolar ducts. One hour of exposure to chrysotile asbestos is sufficient to cause progressive fibrogenesis at alveolar duct bifurcations. The process is initiated by a complement-dependent chemoattraction of lung macrophages to the sites of particle deposition. The central working hypothesis in our laboratory is that these macrophages synthesize and secrete an array of products which mediate the pathogenesis of lung fibrosis. For example, many of the metabolites of arachidonic acid are powerful inflammatory mediators and we have shown that these are synthesized and secreted by alveolar and intravascular macrophages. Also in the past year, we have shown that rat alveolar and interstitial macrophages synthesize and secrete a homologue of human platelet-derived growth factor (PDGF). This factor is a potent mitogen for mesenchymal cells, and we demonstrated that early passage fibroblasts from rat lungs exhibit specific receptors for the PDGF homologue. The degree of macrophage-derived PDGF secretion could be enhanced by treatment of the cells with a variety of stimulants including asbestos fibers, iron spheres, zymosan particles and bacterial lipopolysaccharide. Quantitative analysis of the PDGF was accomplished by eluting macrophage-conditioned medium through a high pressure liquid chromatograph at low pH. This allowed measurement of the PDGF in a competitive binding enzyme immunoassay developed in our laboratory. Studies are ongoing to establish whether or not this growth factor plays a mechanistic role in particle-induced lung fibrosis.

LABORATORY OF REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

LABORATORY OF REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY
Summary Statement

The research goal of the Laboratory of Reproductive and Developmental Toxicology (LRDT) is to understand basic mechanisms underlying normal and abnormal development and reproduction. The research in development concerns germ cell differentiation, genital tract development, ontogeny of hormone response, and developmental regulation of gene expression. Reproductive studies seek to understand the molecular control and biological function of steroid metabolizing enzymes, germ cell biology, and the functional cell biology of estrogens. Included are studies of mechanisms associated with teratogenesis, transplacental hormonal carcinogenesis, and failure of germ cell function. The diversity of scientific disciplines represented in these studies establishes the Laboratory as a unique resource within the NIH. As a component laboratory of the NIH, scientists are cognizant of the human health implications of their basic research and how it may contribute to public health efforts and clinical investigations. The Laboratory is composed of four research groups.

The primary themes under investigation in the Developmental Endocrinology and Pharmacology group are: mediation of estrogen action by growth factors, the developmental control of estrogen responsive genes, target organ specific formation or conversion of estrogens, and mechanisms of induction and expression of estrogen-associated differentiation defects (including neoplasia) in the reproductive tract.

Research by the Gamete Biology group emphasizes identification and characterization of proteins associated with unique structural and functional characteristics of male germ cells, determining if these proteins are synthesized in a stage-specific manner during spermatogenesis, and learning if they are products of genes expressed only during male gametogenesis.

The Pharmacogenetics group studies the molecular basis of the regulation of expression of sex and tissue-specific cytochrome P450 genes. The P450's currently being studied are steroid hydroxylases under the control of pituitary and sex hormones.

Research by the Receptor Biology group involves studying the structure-activity relationships (SAR) of estrogenic chemicals to elucidate the structural basis of estrogenicity, purification and characterization of the murine estrogen receptor, and receptor-mediated cell biology of estrogen action and tissue responsiveness.

Brief summaries outlining the goals and recent accomplishments of some of the research projects conducted in the LRDT follow.

Estrogens are female sex hormones. They are also a class of chemicals which are widely distributed in the environment and to which humans are commonly exposed. Work in our laboratory has sought to understand the mechanisms underlying alterations in genital tract development associated with estrogens. These studies have provided new information concerning the fundamental processes of sex differentiation and the induction of hormone-dependent cancers (such as cervix, uterus, and breast). For example, we recently showed that the mechanisms for processing the information that stimulates cell division or differentiation in immature uterine cells may involve pathways in which the classical estrogen-receptor mediated events are less important. The transduction of the mitogenic signal from estrogen apparently includes synthesis and processing of growth factors such as EGF, IGF-1, and TGFα. As these results are confirmed and expanded, we should gain insights concerning which cells are really at risk for contributing to estrogen-associated cancers. Such findings will have enormous clinical and environmental implications.

Induction of certain genes by estrogens involves the interaction of the hormone with a receptor protein. The specificity and responsiveness of tissues to hormonal stimulation are governed in most part by the presence and biochemical action of this receptor protein. Therefore, we have been studying the purification and characterization of the receptor protein and its intracellular site(s) of action. The protein has been purified (~11,000 fold) from mouse uterus by affinity chromatography. Characterization of the receptor has indicated multiple forms which are proteolytic fragments and not separate gene products. The protease action results in a receptor form which has lost its ability to interact with DNA and, consequently, its biological activity. We have recently shown that the nuclear estrogen receptor specifically exhibits a doublet form when bound by biologically active estrogens. The higher molecular weight component of the doublet is phosphorylated and associated with tightly bound chromatin sites. Weak estrogens or antiestrogens did not produce the doublet form. These findings suggest that this form of the estrogen receptor may be involved in gene activation and hormone responsiveness.

An estrogen-inducible mouse uterine secretory protein has been isolated and its antibody used to isolate cDNA to the messenger RNA. Analysis of the deduced primary structure and additional biochemical characterization indicates that the protein is lactotransferrin. Mammalian transferrin and lactotransferrin constitute a small gene family of iron binding, secretory glycoproteins that have molecular weights of 75-80 kDa and approximately 680 amino acid residues. Each protein shows an intramolecular duplication, and each half of the proteins can bind ferric ions independently. These proteins are also of interest because of their role as growth factors in many cell culture systems and their homology to tumor marker proteins and oncogenes. Lactotransferrin is present in many epithelial secretions and was originally isolated from milk; in addition, it is also found in the intracellular granules of leucocytes. Extracellular lactotransferrin has been observed to have both bacteriostatic and bacteriocidal properties that are only partly mediated by its ability to complex iron ions. The availability of the mouse lactotransferrin cDNA has provided the opportunity to study the lactotransferrin secretion in different tissues in response to different hormone signals and, in addition, compare the structure and functions of the lactotransferrin gene to that of the related transferrin gene family.

A genetic model system was established by which regulation of sex-specific expression of liver microsomal monooxygenase ($\text{I-P-45}_{16\alpha}$) can be studied. The genetic model consists of a trans-acting genetic element (Rip locus) which regulates specific expression of $\text{I-P-450}_{16\alpha}$ in female mice and of the structural gene of the monooxygenase. In the structural gene, a cis-acting genetic element (Ripr locus) was also identified as a site responsible for the estrogen-dependent repression in male mice. The gene for testosterone 15α-hydroxylase ($\text{P-450}_{16\alpha}$ is expressed specifically in male but not in female kidney. On the other hand, the gene is active only in livers of female but not male mice. This reciprocal regulation of $\text{P-450}_{16\alpha}$ in liver and kidney of male mice is controlled by androgen. Sex- and tissue-specific gene regulation will provide a powerful tool for understanding the fundamental mechanisms of gene expression.

Structural development of the sperm is completed within the testis, but the ability to fertilize the egg is gained in the epididymis. Both processes are being studied to understand the mechanisms that regulate male fertility in mammals. The sperm is a highly specialized cell with unique features, such as the flagellum. The fibrous sheath, a cytoskeletal component of the flagellum, helps determine effectiveness of swimming by sperm, and men with abnormalities in this structure are infertile. The fibrous sheath contains germ cell-specific intermediate filament proteins not identified previously and the genes for these proteins will be cloned to determine their structure and role in sperm function. Other studies concern modification of the sperm surface in the epididymis. Sertoli cells surround the spermatogenic cells in the testis and produce a factor in epididymal fluid. It interacts with glycoproteins secreted by the epididymal lining cells to stabilize their binding to the sperm surface. The factor is being characterized to determine if alteration of its production or function could lead to reversible male infertility.

During spermatogenesis undifferentiated germ cells are transformed into highly differentiated spermatozoa. The regulation of gene expression involved in this process has not been well defined. However, several unique germ cell constituents appear at distinct stages of differentiation, suggesting that gametogenesis is controlled by a developmental program. Monospecific antibodies have been used in this study to identify and characterize such constituents, thereby providing additional markers of tissue- and stage-specific events in the testis. In addition, short-term cultures are being used to examine the influence of exogenous agents on isolated spermatogenic cells. These studies have shown that Sertoli cell conditioned medium improves the viability of germ cells *in vitro*, while retinol, required for spermatogenesis *in vivo*, is toxic to cultured spermatids. Such *in vitro* methods may be useful for assessing mechanisms of action of reproductive toxicants and differential sensitivities of various spermatogenic stages to these agents. Understanding the physiological processes which influence spermatogenesis also may provide insights that will be useful in the clinical management of infertility and the development of effective male contraceptives.

When cells are exposed to elevated temperatures, they respond by synthesizing heat-shock proteins (hsp). This response has been observed in organisms as diverse as bacteria and man. Although the specific functions of hsp are unknown, they appear to protect cells against the effects of adverse environmental conditions. We have shown that one of the most abundant proteins (P70) in mouse spermatogenic cells is a unique member of the hsp70 family of proteins. This is the first time a tissue-specific hsp has been identified. Spermatogenic cells, which are actively differentiating into mature spermatozoa, are highly sensitive to even modest elevations in temperature and to many toxic agents. This suggests that P70 may function in germ cell differentiation rather than protecting against the effects of environmental stress. We have also shown that P70 is present in spermatogenic cells, along with the other members of the hsp70 protein family. This is consistent with the recent observations that hsp may have important functions in cell differentiation and embryonic development. Information gained from these studies should be useful in better understanding the effects of environmental agents on male reproduction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70010-11 LRDT

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Normal and Abnormal Embryonic Development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. M. Pratt Head, Experimental Teratogenesis LRDT NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Experimental Teratogenesis

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project terminated. Principal investigator died June 14, 1987.

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70060-15 LRDT

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Developmental Biology/Toxicology of Estrogenic Environmental Chemicals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. A. McLachlan	Head, Develop. Endocrinol. and Pharmacol.	LRDT NIEHS
Others:	R. R. Newbold	Biologist	LRDT NIEHS
	K. G. Nelson	Senior Staff Fellow	LRDT NIEHS
	R. Santti	Visiting Scientist	LRDT NIEHS
	H. Fukamachi	Visiting Fellow	LRDT NIEHS
	C. T. Teng	Senior Staff Fellow	LRDT NEIHS
	N. Bossert	Staff Fellow	LRDT NIEHS
	C. Burroughs	Staff Fellow	LRDT NIEHS

COOPERATING UNITS *(if any)*

Bowman-Gray School of Medicine
Duke University Medical Center
Medical Foundation of Buffalo
University of North Carolina
University of Würzburg

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Developmental Endocrinology and Pharmacology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.0	5	3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies have continued to determine the molecular and cellular targets of estrogenic chemicals and establish the mechanisms by which interactions of estrogens with developing genital tract target cells result in permanently altered differentiation, including dysmorphology and neoplasia. In the period covered by the report, the developmentally estrogenized mouse model has continued to be used to understand both the development of the mammalian genital tract as well as the mechanisms underlying hormonally-associated cancers. In addition to neoplasia in the female structures derived from the Müllerian duct (e.g., uterus), cancers associated with these same embryonic derivatives developed in the prostates of male mice. The ontogeny and tissue specificity of estrogen and androgen metabolism was also studied in the male genital tract. The developing Müllerian duct has been further studied at the cellular and molecular levels. It was determined that the immature mouse uterus, which is an especially sensitive tissue for estrogen-induced cancers, had abundant estrogen receptors (ER) in the underlying stroma, while the epithelium was relatively deficient in detectable ER. This raises the possibility that ER deficient cells may be those which are most susceptible to neoplastic transformation. These same cells however, contain receptors for at least two growth factors--EGF and IGF-1. The immature uterus proliferates in vivo in response to EGF implants suggesting a role for these growth factors. We also recently demonstrated the expression of the mRNA for a uterine secretory protein in the seminal vesicle of developmentally-estrogenized male mice, the first example of pseudohermaphroditism at the molecular level. Studies on the metabolism of estrogens to reactive forms have shown that the mouse uterus forms catechol estrogens and that the enzyme responsible for their inactivation is relatively low. This provides a mechanism for generation of reactive estrogenic metabolites close to the target cell.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70062-01 LRDT

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Role of Growth Factors and Inhibitors in Estrogen-induced Uterine Growth

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. G. Nelson	Senior Staff Fellow	LRDT NIEHS
Others:	T. Takahashi	Visiting Scientist	LRDT NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Developmental Endocrinology and Pharmacology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	1.8	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our goal is to understand the cellular and molecular mechanism by which prenatal and neonatal DES exposure permanently alters uterine cell growth and differentiation. Our approach has been to investigate the role of both positive and negative growth factors in estrogen-induced proliferation of normal mouse uterus. A role of positive acting polypeptide growth factors is suggested by analysis of mouse uterine luminal fluid which demonstrates that high levels of insulin-like growth factor-1 (IGF-1), epidermal growth factor-like peptides (EGF), and lactoferrin are secreted upon estrogen stimulation. Evidence that these factors may act as positive mediators of estrogen-induced growth has been obtained from in vitro studies of mouse uterine cell or organ cultures and from in vivo studies of transplanted pellets containing IGF-1 or EGF under the kidney capsule. In addition to the induction of growth stimulatory factors, estrogen treatment also modified uterine cell response to a growth inhibitor, transforming growth factor-β. Preliminary studies suggest that prenatal or neonatal DES exposure result in a modification of uterine receptors for these growth regulatory factors; such receptor alterations may contribute to the teratogenic and carcinogenic effects of DES. Although our understanding of estrogen action is not clear, estrogen appears to enhance uterine cell proliferation, not only, by inducing multiple growth factors that act in concert to stimulate cell cycle progression, but also, by altering the response of uterine cells to growth inhibitory signals which may amplify the proliferative potential of the cells. Our future plans are to continue to characterize and define the role of peptide mediators of estrogen-induced growth, determine the cell type responsible for the synthesis of these factors, locate the cellular target where these factors act, elucidate whether these factors act alone, synergistically or temporally, control growth, and further define the role of growth inhibitors in modulating uterine cell proliferative response.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70065-12 LRDT

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemical-Receptor Interactions in Reproduction and Hormonal Toxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. S. Korach	Head, Receptor Biology	LRDT NIEHS
Others:	K. Chae	Research Chemist	LRDT NIEHS
	V. Davis	IRTA Fellow	LRDT NIEHS
	J. A. McLachlan	Head, Developmental Endocrinology and Pharmacology	LRDT NIEHS
	F. Ogata	Visiting Fellow	LRDT NIEHS
	S. Yamashita	Visiting Associate	LRDT NIEHS

COOPERATING UNITS *(if any)*
University of Würzburg
Laboratory of Molecular Biophysics, NIEHS
Medical Foundation of Buffalo
Burroughs Wellcome Research Labs
UNC Medical School
Duke University Medical School

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Receptor Biology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	4.0	2.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Estrogen action in reproductive tract tissue involves a mechanism which includes interaction with a receptor protein with subsequent activation and localization in the nucleus. After hormone stimulation, nuclear receptor occupancy followed a bimodal temporal pattern consistent with stimulation of certain tissue responses such as DNA synthesis or enzyme induction. The bimodal receptor pattern was evident in other responsive target tissues such as human MCF-7 cell tumors. DNA synthesis in tumors or uteri can be produced with discontinuous stimulation with a weak agonist and occurs at similar times as the two nuclear receptor events. We have purified (~11000-fold) the mouse uterine estrogen receptor by steroid affinity chromatography and have characterized it by ligand affinity labeling as well as immunoblot analysis with a monoclonal antibody. Studies indicate multiple forms which are proteolytic fragments. Proteolytic processing of the receptor involves a two-step reaction. The first involving a nuclear cysteine protease and the second a soluble enzyme preliminarily characterized as a cathepsin protease. Additional biochemical characterization of the estrogen receptor protein with monoclonal antibodies demonstrated a doublet form differing by 1500 MW. This receptor form was specific to the nucleus and not found in the soluble fraction. The proportion of the doublet varied depending on the biological activity of the compound with potent agonists producing a greater amount of the upper band form. The profile of the nuclear doublet changed during estrogen-stimulation in which the two components were of equal proportion until the time of the second nuclear peak at which time the proportion changed to be primarily the lower band form. Preliminary data indicate both bands are phosphorylated, the upper to a greater extent suggesting this as the protein modification for doublet formation. Experiments to investigate other processes such as acylation or glycosylation are also under study. Receptor modification may be a signal for cellular processing of the protein or programming for its interaction with specific nuclear responsive sites involved in hormone stimulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70067-05 LRDT

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Mechanism of Steroid Hormone in Sex Organ Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. T. Teng	Senior Staff Fellow	LRDT NIEHS
Others:	J. A. McLachlan	Head, Developmental Endocrinology and Pharmacology	LRDT NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Developmental Endocrinology and Pharmacology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	1.2	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mouse uterus has provided a system for the study of estrogen since it contains estrogen receptors and depends on estrogen stimulation for normal physiological functions. We have previously purified an estrogen-induced secretory protein from mouse uterine luminal fluid. Antibody to this estrogen inducible mouse uterine protein has been used to isolate cDNA to the messenger RNA. Analysis of the deduced primary structure and additional biochemical characterization indicates that the protein is lactotransferrin. We have mapped lactotransferrin gene to human chromosome 3 (q21-q23) and mouse chromosome 9 and shown that it was induced by estrogen in a time and dose-dependent fashion in the uterus but not the mammary gland. A high level of lactotransferrin was detected by immunocytochemistry in uterine epithelial cells 1 day after parturition, but immunoreactivity disappeared quickly thereafter. Lactotransferrin message was, however, relatively abundant in the mammary gland at the end of the lactation period. The presence of lactotransferrin in various tissues also was investigated, and two forms of immunoreactive material were detected. A 70kDa band was found in uterine luminal fluid from the estrogen-stimulated immature mouse and in homogenates of lung, vagina, mammary gland, oviduct, spleen, lymph node, and uterus of the adult female mouse. In addition, a 65kDa band was detected in submaxillary gland, kidney, ovary, and all of the above tissues. Brain and duodenum had no detectable immunoreactive material. In future work, we hope to gain some understanding of the factors leading to lactotransferrin secretion by the uterus and mammary gland in response to different hormonal signals. In addition, we will compare the structure and function of the lactotransferrin gene and the related transferrin gene family.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70076-04 LRDT

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Germ Cell-Specific Molecules of Spermatozoa

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. M. Eddy	Head, Gamete Biology	LRDT NIEHS
Others:	R. S. McGee	IRTA Fellow	LRDT NIEHS
	D. R. Joseph	IPA	LRDT NIEHS

COOPERATING UNITS *(if any)*

U. of California, San Francisco
U. of Washington School of Medicine
U. of North Carolina, Chapel Hill
U. of Pennsylvania School of Medicine
Columbia University, Coll. of Phys. & Surg.
The Biomembrane Institute, Seattle
The Hospital for Sick Children, Toronto

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Gamete Biology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The developmental process of spermatogenesis in mammals results in formation of cells highly differentiated in structure and function. The goals are to understand the intrinsic and extrinsic mechanisms that regulate the function of these cells and the stage-specific expression of genes for germ cell-specific proteins. It is hypothesized that such proteins are responsible for unique structural and functional characteristics of sperm and spermatogenic cells. We are using monoclonal antibodies to identify, localize, and isolate such proteins, and will use recombinant DNA methods to clone and characterize their genes. Cytoskeletal proteins of the sperm flagellum have been identified that appear to be germ cell-specific intermediate filament (IF) proteins. Some of these proteins are synthesized in spermatocytes, while others are first present in spermatids. They are recognized by antibodies to IF proteins of somatic cells but also have unique antigenic and biochemical properties. These proteins appear to be products of temporally regulated genes expressed only in spermatogenesis. Other studies are examining the influence of somatic cells on germ cell function and gene expression. In vitro studies have shown that chemotaxis is involved in migration of primordial germ cells (PGC) to the genital ridges. It was also found that an enzyme and an antigen are lost from the surface of PGC after they enter the ridges. In addition, the spermatozoon must interact with the epididymis to gain the ability to fertilize. During this process, the sperm surface is modified by glycoproteins secreted by the epididymal epithelium. These glycoproteins are being isolated and characterized to identify their roles in sperm maturation. Related studies are concerned with purification of a Sertoli cell product which stabilizes binding of some of these epididymal glycoproteins to the sperm surface until near the time of fertilization.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70077-02 LRDT

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Expression of Heat-Shock Genes in Mouse Spermatogenic Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Allen	Staff Fellow	LRDT NIEHS
Others:	E. M. Eddy	Head, Gamete Biology	LRDT NIEHS

COOPERATING UNITS *(if any)*

Division of Toxicology Research and Testing, NIEHS
The University of North Carolina, Chapel Hill
The Hospital for Sick Children, Toronto

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Gamete Biology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The synthesis of heat-shock proteins (hsp) in cells exposed to stress is one of the most highly conserved regulatory systems known and apparently protects cells against the effects of adverse environmental conditions. The process of spermatogenesis is unusually sensitive to slight elevations in temperature and to many toxic agents. However, we have shown that one of the most abundant proteins (P70) in mouse spermatogenic cells is related closely to hsp70, the major inducible hsp. P70 and hsp70 have almost identical mass and isoelectric point. P70 reacts strongly with a monoclonal antibody that is specific for products of the hsp70 gene family. Both P70 and hsp70 are ATP-binding proteins and are purified by using ATP-affinity chromatography. However, P70 and hsp70 are unique proteins on the basis of peptide map analysis and are regulated differently in germ cells. By examining purified preparations of spermatogenic cells, we have shown that preleptotene and leptotene-zygotene spermatocytes contain little P70, while relatively large amounts of P70 are present in pachytene spermatocytes and round spermatids. Labeling studies show that P70 is synthesized primarily in pachytene spermatocytes with little synthesis occurring in other stages of spermatogenesis. The synthesis of hsp70 is not detectable in unstressed cells but is induced in all stages of isolated germ cells following heat stress. These results indicate that P70 is expressed in a stage-specific manner during cell differentiation, whereas hsp70 is only synthesized in germ cells in response to stress. Studies are in progress to examine the molecular biology of hsp in spermatogenic cells and to understand the function of these proteins in the testis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70078-05 LRDT

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Stage-Specific Antigens During Mouse Spermatogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. A. O'Brien	Senior Staff Fellow	LRDT NIEHS
Others:	E. M. Eddy	Head, Gamete Biology	LRDT NIEHS
	M. Maekawa	Visiting Fellow	LRDT NIEHS

COOPERATING UNITS *(if any)*

The University of North Carolina, Chapel Hill
University of Pennsylvania School of Medicine
Columbia University, College of Physicians and Surgeons

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Gamete Biology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Spermatogenesis is a complex process of cell differentiation involving interactions between germ cells, Sertoli cells, and other somatic cells within the testis. One feature of this process is the appearance of several germ cell-specific constituents in a precise temporal sequence. Three areas of research have been pursued to further characterize these unique constituents, both in the acrosome and on the cell surface. (a) Monoclonal antibodies have been used to identify and characterize germ cell components expressed during restricted periods of spermatogenesis. Antibody 1D4 reacts with multiple glycoconjugates that appear in the acrosome of early spermatids but are modified during the late haploid stages so that the determinant is no longer detected. In contrast, this antibody recognizes acrosomal glycoconjugates that are retained in guinea pig spermatozoa. Additional monoclonal antibodies that recognize cell surface constituents have been prepared against proteins excised from two-dimensional gels. (b) Since several glycosylated molecules have been identified on germ cell surfaces and in the acrosome, we have begun to analyze oligosaccharides in isolated spermatogenic cells. Unusual high-mannose oligosaccharides have been identified in both pachytene spermatocytes and round spermatids. Both cell types also have mannose 6-phosphate (M6P) receptors that mediate endocytosis of M6P-bearing ligands. (c) Interactions between Sertoli cells and germ cells at defined stages of spermatogenesis have been examined in short term cultures. When cultured in the presence of Sertoli cell-conditioned medium (SCM), pachytene spermatocytes and round spermatids maintain elevated viabilities and ATP levels. SCM contains multiple glycoproteins and its active fraction has stability characteristics that distinguish it from Sertoli cell growth factors described previously. Germ cell and Sertoli cell constituents, particularly those exhibiting stage and tissue specificity, are candidates for further studies exploring gene regulation and cell interactions during spermatogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 80040-05 LRDT

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Developmental Pharmacogenetics of Microsomal Steroid Hydroxylases*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Negishi	Head, Pharmacogenetics	LRDT NIEHS
Others:	R. Masaki	Visiting Associate	LRDT NIEHS
	T. Ichikawa	Visiting Fellow	LRDT NIEHS
	R. Lindberg	Visiting Fellow	LRDT NIEHS
	H. Yoshioka	Visiting Fellow	LRDT NIEHS
	B. Burkhart	Biologist	LRDT NIEHS

COOPERATING UNITS *(if any)*
None
*Formerly, Developmental Pharmacogenetics of Liver Microsomal Testosterone Hydroxylases

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Pharmacogenetics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.0	5.0	2.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates the mechanism of sex-specific gene regulation of steroid hydroxylases (P-450s) in mouse liver and kidney. Type I $P\text{-}450_{15\alpha}$ is female-specific in liver but male-specific in kidney. Type II $P\text{-}450_{15\alpha}$, on the other hand, is female-specific in both tissues. We characterized genes 15αOH-1 and -2 (-8 Kbp), which encode Type I and II $P\text{-}450_{15\alpha}$, respectively. Despite the differences in sex-specific expressions, both gene structures are nearly identical; there is 96% homology including their flanking and intronic sequences. Although the TATA and CAAT boxes and GRE are located identical positions in the 5'-flanking regions, GC-box-like motif and SV40 enhancer are specific to 15αOH-1 and -2, respectively. The consensus provides a potential sequence by which the genes are differentially regulated by growth hormone and androgen. There are only 11 substitutions in the total 494 amino acid residues between Type I and II $P\text{-}450_{15\alpha}$. We expressed both types of $P\text{-}450_{15\alpha}$ in COS-1 cells and discovered that Type I is the major steroid 15α-hydroxylase, while Type II is the coumarine 7-hydroxylase. We characterized five highly homologous genes, ca, cb, cc, cd, and ce within the mouse male-specific steroid 16α-hydroxylase gene family. Gene ca encode the male-specific 16α-hydroxylase ($C\text{-}P\text{-}450_{16\alpha}$) whose expression is regulated by growth hormone and androgen in liver and kidney. Gene cb, not sex-specific, is expressed only in liver. The gene-encoded P-450 whose protein sequence homology to the $C\text{-}P\text{-}450_{16\alpha}$ is 85%, possesses no steroid 16α-hydroxylase activity. The remaining three genes' expressions are not detected in either tissues. There are several divergent areas in the 5'-flanking regions of all five genes, by which the differential gene expressions might be regulated. We determined that the female-specific $I\text{-}P\text{-}450_{16\alpha}$ gene is -40 Kbp and is a member of a large multigene family consisting of at least 16 genes. We now are investigating the regulation mechanism of this gene by Rip or Ripr locus on mouse chromosome 7.

DIVISION OF BIOMETRY AND RISK ASSESSMENT

DIVISION OF BIOMETRY AND RISK ASSESSMENT
Summary Statement

The Division of Biometry and Risk Assessment (DBRA) plans and conducts basic and applied research in the areas of quantitative and biochemical risk assessment, statistics, biomathematics, epidemiology, and molecular modeling. A major focus of this research effort is the qualitative and quantitative estimation of adverse health effects resulting from exposure to hazardous environmental agents, with particular emphasis on the development of methodology useful in this estimation process. Attention is also directed toward the identification of environmental risk factors and the elucidation of the biological mechanisms that underlie their action. Due to the complexity of many of the issues under investigation, an increasing proportion of this research is being conducted on a collaborative basis, combining the scientific expertise found in DBRA's different organizational units.

In addition to conducting its own research effort, the DBRA also provides statistical, mathematical, data processing, and computer engineering and use support to other programs of the Institute. It assists the Office of the Director in addressing specific health issues that bear on the welfare of the general public, and maintains an active association with peer groups in other federal agencies, academic institutions and private organizations with similar research interests.

The Division of Biometry and Risk Assessment is organized into a Molecular Modeling Section within the Office of the Director, a Statistics and Biomathematics Branch (SBB), an Epidemiology Branch (EB), a Laboratory of Biochemical Risk Analysis (LBRA), and a Computer Technology Branch (CTB). This unique combination of biomathematicians, statisticians, epidemiologists, biochemists, and molecular toxicologists in a single, unified research program permits the multidisciplinary approach that is required to resolve the many complex issues involved in the identification, evaluation, and characterization of environmentally related human health risks.

During the preceding year, DBRA scientists have pursued a number of research issues that have important implications for the identification and characterization of health risks resulting from adverse environmental exposures. These research efforts included the completion of a detailed study of the association between toxicity and chemically induced carcinogenesis in experimental rodents and its implications for the risk assessment process; the continued development, evaluation and application of statistical and mathematical models for characterizing the impact of environmental toxins on different biological processes and health outcomes; the further evaluation of a major congenital poisoning episode in Taiwan involving polychlorinated biphenyls and their contaminants; the investigation of the role of oncogene activation in the onset of lung cancer; and the study of the dependence of chemically induced mutational events on DNA sequence through molecular modeling.

An evaluation of the interrelationship between toxicity, genotoxicity and carcinogenicity in laboratory rodents based on information obtained from two-year animal studies involving 53 positive carcinogens indicated that only 7 exhibited the types of target organ toxicity that could possibly have been the cause of all observed carcinogenic effects. Furthermore, no apparent difference was observed in mutagenicity as measured in Salmonella between "high dose only" carcinogens and the entire set of 53 carcinogens. These findings indicate that any attempt to develop generic regulatory policy for chemical carcinogens based solely on the use of two-year studies to differentiate between "primary" and "secondary" carcinogenicity would very likely be ineffective and/or inaccurate. The more salient, scientific course would seem to be to carefully analyze all chemicals on a case-by-case basis, bringing to bear as much relevant, supplemental biological information as possible.

The development, assessment and use of statistical and biomathematical models for addressing a broad range of scientific issues remains a major research focus of the Division. Most recently, proportional hazard type models have been developed that characterize fertility in terms of the number of menstrual cycles that elapse before pregnancy is achieved and that control for the effects of various modifying factors that bear on this process. Since fertility may be the reproductive endpoint that is most sensitive to environmental insult, the development of statistical models that enhance our understanding of this process has obvious public health implications. Population genetic models continue to be used to investigate the degree of genetic variability that exists in populations and the means by which this variability is generated and maintained. As in the past, a great deal of research effort has also been devoted to quantitative risk estimation models for carcinogenesis. Current interest in increasing the biological relevance of the low-dose extrapolation process had led to the development of more complex models with additional parameters that are presumed to be biologically meaningful as well as estimable from supplemental experimental data. Ongoing research within the Division suggests that the variability associated with risk estimates derived from these models may be substantially increased relative to more traditional models, and that the experimental data typically available for estimating the additional, biologically-based parameters may often be inadequate. Application of quantitative risk assessment models within the Division is best illustrated by the recently completed, extensive modeling of the cancer mortality experience of the Hiroshima and Nagasaki atomic-bomb survivors cohorts to obtain site-specific risk estimates for the soon to be released National Academy of Sciences report on the biological effects of ionizing radiation. Finally, computer-based molecular modeling, which has the potential to become an important alternative to the use of experimental animals in some instances, is being used to evaluate theoretical factors involved in mutagenesis and in the initial steps of carcinogenesis.

The investigation of the 1979 poisoning that occurred in Taiwan as a result of using cooking oil contaminated by thermally degraded polychlorinated biphenyls has continued. Because these chemicals persist in human tissue, children born to female patients after the outbreak were exposed in utero. One hundred seventeen children born to affected women along with unexposed controls have been examined and evaluated. The exposed children were found to be shorter and lighter than the controls; and they had abnormalities of gingiva, skin, nails, and lungs more frequently than did controls. The most recent findings indicate that exposed children show delay of developmental milestones, deficits on formal developmental testing, and abnormalities on behavioral assessment. These observations are most consistent with a generalized disorder of ectodermal tissue, and represent a syndrome that is one of the very few to result from transplacental exposure to pollutant chemicals. This study is certainly of interest in its own right, and it may also serve as a model for assessing the potential effects of these important pollutants at low environmental dose levels.

Ongoing research within the Division has developed and utilized a number of new, more sensitive assays for the detection of activated oncogenes in neoplastic tissue. For example, a recently completed study of human lung tumors detected a much higher percentage of activated oncogenes than had previously been reported. Specifically, 7 of 8 human pulmonary adenocarcinomas and 2 of 3 squamous cell carcinomas examined contained activated oncogenes: 4 were activated K-ras, one was raf, one was Ha-ras and the remaining 3 were of unknown origin. Identification of specific genes involved in the onset of human lung cancer may have important implications for the development of therapeutic regimens and, potentially, for the prevention of this devastating disease. Furthermore, since the activation of the K-ras oncogene is a common occurrence in rodent pulmonary adenocarcinomas, these findings reaffirm the value of the experimental rodent system both as a model for cancer chemotherapeutic studies and as a basis for estimating human cancer risk. Future research will concentrate on the identification of the uncharacterized oncogenes detected in both human and rodent pulmonary squamous cell carcinomas and adenocarcinomas.

During the past year the newly initiated program in computer-based molecular modeling has made a great deal of progress. Software tools have been accumulated for performing precise quantum mechanical calculations that give the geometries, electronic structure and charge distributions on small molecules; for calculations of less precise but still useful mechanical properties such as geometry and binding energies of DNA or proteins; for calculations of the dynamic properties of short strands of DNA or proteins; and for calculations of solvation and counterion effects in site-directed mutagenesis "experiments". These tools are being and will continue to be employed in a number of research projects addressing different issues relevant to the understanding of the molecular details of mutagenic and, ultimately, of carcinogenic events.

In addition to its own research activities, the Division of Biometry and Risk Assessment supports and, in some instances, collaborates in a broad range of research projects conducted under contract or interagency agreement. For example, the International Agency for Research on Cancer, supported by the DBRA, has essentially completed the establishment of an International Register of individuals who were exposed to phenoxy acid herbicides and their contaminants in a workplace setting or who serve as controls for those who were exposed. This Register, which has already enrolled over 18,000 workers, represents a unique, worldwide source for investigating the health effects of phenoxy acid herbicides, such as those used in the production of "Agent Orange," through epidemiologic follow-up studies. DBRA contractual research is also illustrated by the recently initiated case-control study of the relationship between indoor radon pollution, cigarette smoke exposure and cancer. While it has been estimated that indoor radon pollution may be responsible for as many as 20,000 lung cancer deaths a year in the United States, its specific impact is difficult to ascertain because of the possibly confounding effect of exposure to cigarette smoke. Therefore, this study will attempt to characterize the potential interaction between exposure to indoor radon pollution and cigarette smoke, and to assess the effect of passive or indirect smoking on indoor radon pollution as well.

Future research efforts within the Division will focus on a number of important environmental health issues, many of which are relevant to improving the scientific basis underlying the risk assessment process. These research activities will include experimental and epidemiologic studies of the molecular events involved in the multistage process of carcinogenesis, such as the activation and expression of oncogenes and the identification of factors that regulate cell proliferation. Computer-based molecular modeling will also continue to be developed to understand more clearly chemically induced mutational events in DNA and altered protein structure and function; and statistical studies will be used to characterize the uncertainty associated with the modeling of low-dose cancer risks and to refine risk quantification for other endpoints of interest in areas such as reproductive and developmental toxicology and immunotoxicology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 43010-03 DBRA

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Macromolecular Modeling and Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David G. Hoel	Director	DBRA/OD	NIEHS
Others:	Marshall W. Anderson	Research Chemist	DBRA/LBRA	NIEHS
	Tom Darden	Sr. Computer Specialist	DBRA/OD	NIEHS
	Lee G. Pedersen	Research Chemist	DBRA/OD	NIEHS
	Charles Foley	Staff Fellow	DBRA/LBRA	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Division of Biometry and Risk Assessment

SECTION

Office of the Division Director

INSTITUTE AND LOCATION

NIH, NIEHS, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ **(a) Human subjects** ☐ **(b) Human tissues** ☒ **(c) Neither**
  ☐ **(a1) Minors**
  ☐ **(a2) Interviews**

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is concerned with determining theoretical factors involved in mutagenesis and in the initial steps of carcinogenesis. The proliferation of new experimental techniques in genetic engineering is providing innovative pathways for studying the dependence of chemically induced mutational events on DNA sequence. Computer modeling is being used to examine the physical chemical factors (charge distributions, binding energies, stereo-chemistry, activation energies, solvation, counterions) contributing to site specificity of DNA damage by chemical agents. The same techniques are also being employed to determine changes in molecular properties of oncogene proteins as a consequence of specific mutations.

Specifically, computer intensive quantum mechanical calculations are employed to determine the properties of small molecules. These results are then used to paramaterize empirical force fields that can in turn be used to study the mechanical properties of large molecules such as meaningful segments of DNA and proteins, e.g., by the analysis of computer graphics.

Research issues of ongoing interest include the characterization of local structures of DNA sequences (native and chemically modified) that contain known hotspots from mammalian oncogenes and bacterial systems, the examination of the molecular details of the initial attack by mutational metabolities, and the understanding of the consequences of single amino acid changes on the function of critical proteins such as the p21 ras oncogene protein.

COMPUTER TECHNOLOGY BRANCH

COMPUTER TECHNOLOGY BRANCH
Summary Statement

As well as developing overall strategies for computing at the Institute, the Computer Technology Branch (1) manages and operates the Institute's central VAX computer system including hardware development and systems programming; (2) coordinates data communications and networking activities associated with the central system, workstations, and remote facilities such as NIH/DCRT; (3) develops laboratory computers to support Institute research; (4) develops administrative information systems (application software) for the Institute; (5) develops scientific information systems for the NTP; (6) provides services to VAX users including consulting, problem investigation, training, manuals, and information distribution; (7) manages the Institute's word processing, office automation, and workstation activities including personal computers; and (8) manages the formal administrative systems associated with computing at the Institute.

Management of the central VAX computer facility includes hardware development, systems software development, and traditional operations functions for the VAX processors and peripherals including management of computer accounts, supplies, storage media, and disk space. Hardware design, procurement, installation, and maintenance are managed for computer equipment, power, air conditioning, and physical security. As part of the systems programming effort VAX performance is monitored, resource utilization is monitored, capacity needs are forecast, resources are reallocated, and the VMS operating system is tuned for improved performance as the computing resources and workload change.

Data communications support consists of the provision (analysis of requirements, design, selection, installation, and maintenance) of all hardware and software components necessary to fulfill the intra- as well as extra-Institute data transmission requirements of all Institute employees and contractors. A major task continues to be responding to numerous requests for information and assistance regarding the proper configuration and operation of various terminals, microcomputers, printers, modems, and related equipment. Major projects recently completed include the implementation of access to BITNET from the central VAX facility and access from the TDMS laboratories to the VAX.

Laboratory computing support is provided to the Institute with a primary emphasis on consulting with researchers to determine functional needs and on performing risk/benefit analyses of possible applications of computers in the laboratory. The traditional effort of laboratory data acquisition continues with the design, implementation, and maintenance of five computer-assisted data acquisition and analysis systems for serially-interfaced radiometric analyzers. The evaluation, design, and implementation of scientific software supporting researchers' specific needs has continued, most notably with DNA sequencing software. The use of standard approaches, such as MICRO/PDP11 computers for data acquisition and RS/1 software for data analysis, has continued to simplify and streamline development and support for researchers with common needs.

The Information Systems Group has responsibility for information systems project development for the Institute. The Information Systems Group manages 40 projects, assists users in developing their own systems, develops automated systems for the various Institute Divisions, develops automated systems for the

Office of Management and for the Facilities Engineering Branch, and provides Institute support for various information systems software. The Information Systems Group also evaluates and implements software engineering tools. These tools include both data management software as well as computer aided systems analysis and design software. The Information Systems Group is further required to provide analysis and planning for computer system integration with NIH and DHHS. In the past year, ISG has overseen the delivery of a new travel system, an ADP equipment tracking system, and a centralized personnel database. ISG has also overseen the conversion of several peripheral systems utilizing the centralized personnel database. ISG has also selected and participated in testing of products that will improve and shorten the software development process at NIEHS.

In FY89, efforts will be made to combine systems and processes to improve reports available to NIEHS management and reduce the time necessary to conduct ADP oriented processes. The goal will be to modify NIEHS systems so that on item of data will be entered only once and then automatically updated in each system that requires that information. Improved procedures for reporting and tracking the budget will be designed and implemented both for local NIEHS systems as well as NIH and DHHS systems. Projects will be initiated to take advantage of other emerging technologies such as Local Area Networks. There will be further evaluation and purchase of tools in the area of automated report and program generation, software optimizers, and data base tools.

Scientific information systems for the NTP include the Chemical Information and Tracking System (CHEMTRACK), the Carcinogenesis Bioassay Data System (CBDS), and the Toxicology Data Management System (TDMS). CBDS accomplishments include continued support of the NTP carcinogenicity bioassay studies with the generation of over 30 chemical package reports consisting of tumor and non-tumor pathology tables, survival curves and weight gain tables for the NTP Technical Reports. The Chemtrack information system was converted from the Parklawn Computer Center where it ran under the Model 204 DBMS to the NIEHS VAX running under RdB. Software was developed to allow NTP management to interactively query the Chemtrack system. TDMS was converted to the ADABAS Data Base Management System and also converted to run on a VAX 8650 at NIEHS. Nearly 5000 modules consisting of more than 500,000 lines of code were converted or rewritten. In FY88, TDMS was used to process data for more than 150 chemical compounds during in-life and/or pathology stages. Most chemicals were tested on two species and in some 90 day studies by multiple routes of exposure. Both in-life and pathology data are routinely updated and hundreds of reports are produced each month as needed by the laboratories and NTP scientists. With the award of a microcomputer data base system in late FY88, revisions to the existing microcomputer based data collection, processing and analysis systems will begin. Support and further development of data bases for Salmonella, Mouse Lymphoma, Cytogenetics, and Drosophila information continued.

In FY88 the User Services Group continued to provide a central point of contact for the NIEHS computing community for information and documentation, help in problem solving, consulting, training, testing software, and other miscellaneous support functions. A user area has been provided with various workstation hardware and software for general use by the computing community. The lead user concept was advertised and tested with implementation planned for late FY88. This involves identifying personnel in user areas to serve as a focal point for questions regarding the VAXcluster, personal computers, training of staff members, and communicating information to the user community. It is patterned after a structure in use at NIH. The monthly newsletter, CONNECTIONS, continued to be published and significant progress was made on an NIEHS Computing Manual which describes software, equipment, and services available to NIEHS employees.

Training for BITNET was offered and VCR tapes for the SAS (Statistical Analysis System) software package were ordered to enhance VAX-based training available to Institute users. This supplements the courses already offered on Introduction to the VAX, EDT, and VAXmail. The Rally development tool was adopted for evaluation. Training was provided to CSC and CTB application developers and support was provided for the first Rally applications developed throughout the Institute. Preliminary evaluation indicates that use of this tool will reduce the time necessary to develop some software. USG developed, tested and began implementation of the Problem Log System designed to receive, process, and quantify user requests. Other activities involved testing new AT&T modems, developing a comprehensive printing scheme for the Institute, planning improvements to our BITNET service, and improving information dissemination for all Institute employees.

In FY88, an evaluation of the All-In-1 office automation software was planned, conducted, and analyzed. All-In-1 contains the WPS word processing software, improved electronic mail, and other software to perform office automation functions. This software, the concept of lead users, and the CSC training were evaluated in four of the scientific groups of the Institute. Continued evaluation of software to further integrate with All-In-1 for document exchange is being done. Two packages for converting documents from one software package to another are being compared with results to be published in late FY88. Whether to use the VAXcluster for centralized word processing and office automation software will be determined after this study is completed.

Support continued for word processing users on the VAX, on DEC PCs, and on CPT stand-alone word processors. Assistance was provided for Institute secretaries who upgraded from their CPT 8525 to Phoenix Juniors. A technical evaluation of word processing packages for the IBM PCs was completed and WordPerfect was recommended. This is compatible with recommendations at NIH.

The workstation group prepared documents that allowed users at the Institute to order a variety of personal computers. The group consulted with users to ensure the best overall system for the application was chosen, then provided individual consultation for new users. Commercial training was arranged for courses on MS DOS and WordPerfect in conjunction with the Personnel Management Branch. Evaluation of software packages for the IBM PS/2 was completed and a list of recommended software was provided along with the ordering information. ZSTEM was selected as the terminal emulation package and additional code was developed to better use ZSTEM with the Institute's VAXcluster and communication with external IBM systems. Routines were also developed to automatically install the recommended software for the users.

The Computer Technology Branch utilizes an interagency agreement with the General Services Administration to acquire computer and related expertise. Directed and monitored by five CTB personnel, seventy on-site contract personnel provided computer service to NIEHS in computer operations, systems analysis, programming, office automation, and data entry. Procedures are underway to award a data processing support contract to provide these skills; proposals are anticipated late in FY88 with award anticipated in early FY89.

The branch's project management system was implemented in the remaining areas of the branch. All types of contract work, including operations, systems programming, information systems development, scientific computing, office automation, and data entry, have been defined into projects and managed through use of the unified project management system.

EPIDEMIOLOGY BRANCH

EPIDEMIOLOGY BRANCH
Summary Statement

Overview and Objectives

The Epidemiology Branch carries out research with human subjects and human data. We emphasize innovative topics, interdisciplinary collaboration, and laboratory methods. These studies are only feasible within a research institution like NIEHS, since the variety of disciplines available for collaboration coupled with the shared interest in environmental toxicology are not found elsewhere. There are three general areas of research: reproduction and early development, chronic disease, and oncogenes. Reproduction, broadly defined, is both a process of basic science and public health interest in its own right and a plausible target for environmental toxins. Chronic diseases such as cancer, heart disease, and renal disease are among the major killers as well as causes of major morbidity in all developed countries. Environmental causes of cancer have been fruitfully studied for decades, but relatively little attention has been paid to the possible environmental causes of other kinds of chronic diseases. While we have done and will continue to do cancer studies, we devote considerable effort to other important chronic diseases. Finally, the role of oncogenes in human cancer is a new area that shows promise for exciting avenues of research. There is potential for the use of assays of activated oncogenes and measures of susceptibility to oncogene activation in studies of cancer etiology and prognosis. If the potential is realized, this technology may make much more explicit the role of environmental and work place chemicals in the cause of human cancer, and also identify which individuals are most susceptible to such damage.

Reproduction and Early Development

Early pregnancy loss, that is, loss that occurs before a woman knows she is pregnant, has until recently been undetectable in humans. We have completed a prospective study of very early pregnancy loss, in which we have measured human chorionic gonadotropin in daily urines from women who are trying to become pregnant. A newly-developed hormone assay allows the risk of early loss to be measured accurately in humans for the first time. Twenty-two percent of pregnancies in our study ended in unrecognized loss. Effects of environmental factors on the risk of such loss will be explored. Another area of study has been human fecundity, and its sensitivity to disruption by environmental exposures. For example, we have found that women whose mothers smoked while pregnant with them take longer to become pregnant as an adult. Also, we found an unexpectedly strong relationship between caffeinated beverage consumption and time to clinical pregnancy. These findings suggest that of all reproductive endpoints, fecundity may be one of the most sensitive to damage from environmental toxins.

A second approach to reproductive studies has been to study populations that have been accidentally exposed to high levels of a toxin, or to use large numbers of observations and sensitive methods in studies of relatively low exposures. An accidental poisoning over 2000 people in Taiwan presented an opportunity to study the offspring of poisoned mothers. One-hundred and seventeen exposed children and 108 controls were examined in the field. The exposed children have higher rates of respiratory disease, dental, skin, and pigment abnormalities, and developmental delay, and a few have mild hepatic porphyria. We plan to examine specific aspects of the syndrome, such as dermatology, neurology, and development, in more detail. In the US, we have studied almost 900 children exposed to PCBs and DDE, a form of DDT, transplacentally and through contaminated breast milk. Transplacental exposure to PCBs appears to result in small decrements on developmental testing at ages 6 and 12 months, while subsequent exposure through breast milk has no effect. We have longitudinal developmental data on the children out to age five, and we plan to see whether the effects persist. Since exposure to PCBs and DDE is very common, and since both have been studied extensively in the laboratory, understanding the results of exposure has both public health significance and aids in the interpretation of laboratory data.

Chemicals that are weakly estrogenic are widespread in the environment, including several pesticides and a variety of chemicals in plants. It is not clear, however, whether these plant and other estrogens are biologically active in human beings at reasonably attainable doses. As a first step in exploring this question biological effects of plant estrogens are being measured in postmenopausal women. Postmenopausal women produce little or no estrogen of their own, and are thus a model for studying the effects of exogenous estrogens. Seventy volunteers were recruited for the study. They ate a diet high in soybeans, a food rich in plant estrogens. Urine, blood, and vaginal cells were collected before and after the diet for measurement of several biological factors expected to change with low doses of estrogens. Analysis of the data from this study is now underway.

Chronic Disease

Our approach to the study of chronic diseases includes both the study of persons with disease and the study of populations exposed to potential environmental hazards. For example, we have completed two case-control studies that evaluated the importance of several exposures suspected as causes of chronic renal disease. In a large multi-center study, renal disease risk was found to be substantially elevated among persons who consumed analgesic medications containing phenacetin or acetaminophen on a daily basis. While an association with phenacetin and some forms of renal disease has been reported in the past, studies in the U.S. have been largely negative. An association with acetaminophen use has been postulated because acetaminophen is a major metabolite of phenacetin, but this has not yet been reported. Patients with glomerulonephritis, a specific form of chronic renal disease, were more likely to report exposure to solvents. Patients with IgA nephropathy were more likely to report frequent colds and use of antibiotics, and, unexpectedly, were much more likely to report use of artificial sweeteners. We plan additional analyses of these data to look at other drugs and occupations that might increase renal disease risk, and to quantify the magnitude of risk associated with hypertension, diabetes, and family history of these and other diseases.

The health consequences of active cigarette smoking are well known, but the hazards to persons passively exposed to the smoke of others is a question that is both of current interest and difficult to study. Using data on smoking by household members that had originally been obtained in 1963, we have examined all-cause mortality and cancer incidence through 1975 in nonsmokers, passive smokers, and active smokers. We have found that the overall mortality of passive smokers was midway between that for nonsmokers and that for active smokers. Much of the increased mortality observed for passive smokers was due to heart disease, but deaths from all causes were still significantly increased. Of particular interest was an observed increase in risk for chronic lung disease among nonsmoking women who lived with smokers that confirms another recent report. Studies of passive smoke exposure are limited by our ability to quantify exposure adequately. As with the study of other very low dose exposures, effects from passive smoking are difficult to distinguish from other characteristics of persons exposed to cigarette smoke. By continuing to pursue this area of research, new approaches that can also be applied to other low dose exposures may emerge.

Exposure to radon-222 has been associated with excess risk of lung cancer in underground miners and the general population. Although the lung is the primary target organ for radon, other parts of the body may be exposed to circulating radon and decay by-products. We studied cancer mortality and measurements of groundwater radon concentration in two states, North Carolina and Maine. There was no clear relationship between radon exposure and cancer mortality at sites other than lung in adults; in children, there is some evidence that sites other than lung may show some relationship to water levels of radon.

The acute leukemias appear to be comprised of many different diseases with specific immunologic and cytogenetic features, but with common clinical appearance. Early research suggests that prognosis and treatment response vary with cytogenetic profile and that different leukemogenic exposures might be associated with risk for specific cytogenetically defined subgroups of leukemia. In a multi-center case control study of patients with acute lymphocytic and acute non-lymphocytic leukemia, a variety of environmental and occupational exposures potentially related to leukemia risk are being explored. This study is unique in that patients are being classified according to the presence or absence of particular clonal chromosome abnormalities in their bone marrow. Over 470 patients and 130 controls have been enrolled to date. Interim analysis of these data suggests that chromosome classification should enhance our ability to detect certain environmental risk factors.

Oncogenes

Oncogenes are a class of abnormal genes capable of inducing cancer. They are derived from normal genes, called proto-oncogenes, which are present in all cells. In animals, chemical carcinogens can change proto-oncogenes into oncogenes through mutation. Smoking and environmental exposure information is being collected on 30 patients about to undergo resection of bladder tumors. The patient's exposure will be correlated and compared with the results of oncogene assays done on portions of their resected tumor. In a second study, oncogene assays will be done on bladder tumor tissues resected from 100 individuals with occupational exposures to the potent bladder carcinogens benzidine and ß-napthylamine; and compared to oncogene assays done on bladder tumor tissues resected from 100 individuals without such exposures. If chemically induced tumors differ from "background" tumors in terms of their oncogene activation, then much more precise etiologic studies will be *possible*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-ES-43002-12 EB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Human Exposure to Halogenated Aromatic Compounds

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Walter J. Rogan	Chief	EB	NIEHS
Others:	Beth C. Gladen	Statistician	SBB	NIEHS

COOPERATING UNITS *(if any)*
Statistics and Biomathematics Branch, NIEHS

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Polychlorinated biphenyls (PCBs) and the DDT family are toxic, widespread hydrocarbons that are poorly understood in terms of their toxicity for human beings. In addition to their direct toxicity, both of these chemicals pass from mother to child through the placenta and by contaminating breast milk. This project includes a study of subjects exposed to low levels of these compounds in the US and a study of subjects exposed to higher doses in Taiwan. The Breast Milk and Formula study is a birth cohort follow up study of 856 North Carolina children. PCBs and DDE (the stored metabolite of DDT) are measured in breast milk and the children are followed medically over time. All children have completed 5 years of observation and are now followed only by a birthday card registry. Higher exposure to PCBs while the children were in utero correlate with lower developmental scores at ages 6 and 12 months; there is no additional effect from exposure through breast milk, and no persistent effect with DDE exposure.

An epidemic of 2000 cases of PCB poisoning occurred in Taiwan in 1979. Rice oil was accidentally contaminated during manufacture. We did a survey of 117 children who were born to mothers who were poisoned, 40 of their older siblings, and 107 controls. All children received a physical examination and the mothers answered a questionnaire about their children's health. A few of the children have mild hepatic porphyria, and in general the transplacentally exposed children show a syndrome most consistent with an acquired ectodermal dysplasia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-1-ES-43004-10 EB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Environmental Exposures and Chronic Renal and Other Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Dale P. Sandler	Epidemiologist	EB	NIEHS
Others:	Walter J. Rogan	Chief	EB	NIEHS

COOPERATING UNITS *(if any)*
Bowman Gray School of Medicine/Baptist Hospital, Duke University Medical Center, University of North Carolina Medical School, Charlotte Memorial Hospital

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.35	0.35	0.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Environmental exposures may play an important role in a number of poorly understod chronic diseases. One such disease, chronic renal failure, affects more than 50,000 individuals in the United States who require maintenance dialysis or transplant at a cost of more than $1.5 billion per year. Despite the magnitude of this health problem, chronic renal disease has received little attention in epidemiologic studies.

Two case-control studies have been completed that evaluate the importance of several exposures that have been suspected to be causes of chronic renal disease. In one study, over 500 patients with newly diagnosed chronic renal disease and over 500 population controls were surveyed by telephone to obtain exposure histories. Patients with renal disease reported more use of analgesic medications for a variety of specific symptoms, and were substantially more likely than controls to report regular use of analgesics. Renal disease risk was significantly increased for regular users of analgesics containing phenacetin (OR=6.5) or acetaminophen (OR=6.9). Results were not explained by other factors associated with analgesic use, and risks increased with increasing doses of these drugs. While there was no overall association between solvent exposure and renal disease, patients with glomerulonephritis were about twice as likely as other renal disease patients or controls to report jobs involving potential exposure to a variety of solvents and were significantly more likely to report exposure to any named solvent. In preliminary analyses, these solvent-related findings do not appear to be explained by confounding by other factors. The second study explored risk factors for IgA nephropathy. Preliminary results suggest that patients with IgA disease were more likely to report frequent colds and use of antibiotics. Patients were also more likely to report use of artificial sweeteners. Analysis of both studies is ongoing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 43008-08 EB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical and Cellular Environmental Epidemiology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Richard B. Everson Medical Officer EB NIEHS

COOPERATING UNITS (if any) Columbia University, U.S. Department of Agriculture Western Regional Research Center, University of North Carolina at Chapel Hill, Duke University, Baylor College of Medicine, Laboratory of Biochemical Risk Assessment, NIEHS, Laboratory of Pharmacology, NIEHS

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS: PROFESSIONAL: OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

PROJECT TERMINATED: SEPTEMBER 30, 1987

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-ES-44003-11 EB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Epidemiologic Study of Reproductive Outcomes and Environmental Exposures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Allen J. Wilcox	Medical Officer	EB	NIEHS
Others:	Donna D. Baird	Senior Staff Fellow	EB	NIEHS
	Beth C. Gladen	Statistician	SBB	NIEHS
	Clarice R. Weinberg	Statistician	SBB	NIEHS

COOPERATING UNITS *(if any)* Developmental Endocrinology Branch and Biometry Branch, National Institute of Child Health and Human Development, National Institute of Dental Research, Columbia University, Atlanta University, University of North Carolina, University of Bergen, Norway.

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	2.1	0.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The reproductive epidemiology project emphasizes the development and application of methods for measuring human reproductive damage. Reproductive damage includes infertility, subclinical pregnancy loss, clinically-recognized pregnancy loss (or spontaneous abortion), impaired fetal growth, and low birthweight. Each of these outcomes can be caused by environmental factors, and each represents a possible endpoint for detecting effects of toxins on human health. This year we have completed our prospective study of subclinical pregnancy loss among a group of 221 North Carolina women who were attempting pregnancy. These women collected daily urine specimens for up to six months. Using a very sensitive assay for the hormone hCG, we identified 198 pregnancies at about 8 to 10 days after fertilization. 22% of these pregnancies failed to last long enough to be recognized by the woman or her physician. If we also include losses that occurred to recognized pregnancies, then 31% of all assay-detected pregnancies failed to survive to term. We are analyzing these data further for evidence of any effects of common exposures (such as caffeine or alcohol) on the risk of having a pregnancy loss. We are planning a new study of early pregnancy loss among women occupationally exposed to a reproductive toxin such as lead, in order to test how useful this endpoint might be in environmental studies. The pregnancy study also offers data on factors that influence a woman's capacity to conceive. For example, we have found a strong relation between caffeine consumption and lower fertility. Work has progressed in other areas as well. In collaboration with Norwegian scientists we have made progress in developing methods for the analysis of birthweight and infant mortality, using the Norwegian Medical Births Registry.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 46002-04 EB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Environmental Exposures and Cancer Risk

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Dale P. Sandler	Epidemiologist	EB	NIEHS
Others:	Gwen W. Collman	Senior Staff Fellow	EB	NIEHS
	Eric S. Johnson	Visiting Scientist	EB	NIEHS

COOPERATING UNITS *(if any)*

University of Minnesota, Harvard University, Cancer and Leukemia Group B member institutions

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

TOTAL MAN-YEARS: 1.8	PROFESSIONAL: 1.8	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A significant proportion of the leukemias, particularly acute nonlymphocytic leukemia (ANLL) may be due to environmental or occupational exposures. ANLL and acute lymphocytic leukemia (ALL) may each be comprised of many different diseases with specific immunologic and cytogenetic features, but with common clinical appearance. There are data to suggest that risk factors may be specific to subgroups of ANLL and ALL defined by these features. A study is being conducted to address the potential for enhanced risk factor detection in well-characterized subgroups of leukemia patients. The study was motivated, in part, by clinical reports suggesting that leukemia patients with specific chromosome changes in bone marrow were more likely than other leukemia patients to have had prior chemotherapy or occupational exposure to chemicals. Using a case-control design, as many as 550 adults with ANLL and 150 adults with ALL who enroll for treatment in first line treatment protocols sponsored by a cooperative cancer treatment group are being studied to evaluate the leukemia risk associated with a variety of environmental and occupational exposures. Patients with leukemia are identified at diagnosis and interviewed by telephone while they are still hospitalized regarding exposure to solvents and chemicals, active and passive smoking, use of hair dyes, irradiation, and family history of certain diseases. Healthy comparison subjects chosen by random telephone screening are also interviewed. Cytogenetic studies being done by the cooperative group are used to classify patients according to the presence or absence of particular chromosomal abnormalities, and risk factors for specific subgroups of patients will be evaluated. Risk factors will also be correlated with other clinical and laboratory parameters such as FAB classification and immunologic phenotype. Over 470 patients and 130 controls have been enrolled to date.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-47001-02 EB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Exposure to Radon-222 and Cancer Mortality

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Gwen W. Collman	Senior Staff Fellow	EB	NIEHS
Others:	Dale P. Sandler	Epidemiologist	EB	NIEHS
	Dana P. Loomis	Student	EB	NIEHS

COOPERATING UNITS *(if any)*

University of Maine, Orono, Maine

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.05	0.65	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Exposure to radon-222 has been associated with excess risk of lung cancer in underground miners and the general population. Radon enters the body primarily through inhalation and ingestion. Although the lung is the primary target organ for radon, other parts of the body may be exposed to circulating radon and decay by-products. The role of radon in the development of cancers other than lung cancer is not well studied, especially in children. The effects of alpha radiation exposure during pregnancy and early childhood are not known. We studied the risk of various selected types of cancer using mortality data collected between 1978-1982 and measurements of groundwater radon concentration previously collected in two states, North Carolina and Maine. In adults, cancer sites of interest were cancers of the upper respiratory tract, cancers of the gastrointestinal tract, the leukemias, bone cancer and female breast cancer. In children aged less than 15 years, selected cancers included leukemia, brain cancer, soft tissue sarcomas, and bone cancer. County mean radon values in North Carolina were calculated and ranged from 0 to 10,692 pCi/l. These values were divided into exposed and unexposed counties. Age-sex-adjusted mortality rates were calculated for each cancer site in each radon group. Studies are nearing completion in North Carolina and are ongoing in Maine. We were unable to find any consistent associations between radon and cancers other than the lung in adults. Preliminary results from the study of childhood cancers suggest that all cancers combined and childhood leukemia may be related to radon exposure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-ES-47002-02 EB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biological Effects of Plant Estrogens in Postmenopausal Women

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Donna D. Baird	Staff Fellow	EB	NIEHS
Others:	Allen J. Wilcox	Medical Officer	EB	NIEHS
	Clarice R. Weinberg	Mathematical Statistician	SBB	NIEHS
	John McLachlan	Chief	LRDT	NIEHS

COOPERATING UNITS *(if any)*
Laboratory of Reproductive and Development Toxicology; Statistics and Biomathematics Branch, NIEHS

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Exposure to highly estrogenic substances can disrupt reproduction and increase cancer risk. Effects on bone metabolism and cardiovascular health also have been documented. Chemicals that are weakly estrogenic are widespread in the environment, including several pesticides and a variety of chemicals in plants. Health effects of these environmental estrogens are not known. As a first step in exploring this question, biological effects of plant estrogens will be measured in postmenopausal women. Ninety-four volunteers were recruited for the study, 68 of whom ate a diet rich in plant estrogens, and 26 of whom ate their usual diet. Urine, blood, and vaginal cells were collected for measurement of pituitary hormones, liver-produced plasma proteins, and estrogenization of vaginal cells. The field work is complete and specimens are currently being analyzed. If plant estrogens are found to be biologically active in postmenopausal women, further questions arise: (1) What effects do these chemicals have on other segments of the population: males, reproductively active women, and babies on soy formula? (2) Do effects of plant estrogens explain some of the differences in morbidity and mortality seen in vegetarians compared with nonvegetarians? (3) Can dietary changes be used in prevention or treatment of estrogen-related conditions? (4) Do other environmental estrogens (like pesticides) affect human health through estrogenic changes in human biology?

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-48003-02 EB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Etiologic Significance of Oncogene Activation in Human Bladder Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jack A. Taylor	Staff Fellow	EB	NIEHS
Others:	Marshall W. Anderson	Research Chemist	LBRA	NIEHS

COOPERATING UNITS *(if any)*

National Cancer Institute, Johns Hopkins University, University of North Carolina, University of Wisconsin, Roswell Park Memorial Institute, Salem County Memorial Hospital

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Oncogenes are frequently found in a variety of human cancers. Studies in the Branch have been established to investigate the role of oncogenes in the etiology of carcinogen-induced human tumors. Bladder cancer was chosen as a model tumor for this investigation, since it is perhaps the best prototype of a chemically induced human tumor. In one study, smoking and environmental exposure information is being collected on 30 patients about to undergo resection of bladder tumors. Tumor tissue, blood and urine samples and questionnaire data have been collected on approximately 10 patients to date. Assays for oncogenes, such as transfection into NIH 3T3 cells and nude mice assays should begin shortly.

In a second study, oncogene assays will be done on bladder tumor tissues resected from approximately 60 individuals with occupational exposures to the potent bladder carcinogens benzidine and ß-napthylamine; and compared to oncogene assays done on bladder tumor tissues resected from 100 individuals without such exposures. Individuals with exposure to these chemicals are rare, but samples of bladder tumor tissue from them are available in the form of paraffin-embedded tissue blocks made and stored following surgery. Recently, oncogene assays have been developed for use with these blocks, allowing us to determine the oncogene status of tumors resected many years ago from individuals with exposures to these powerful chemical carcinogens. All paraffin blocks have been retrieved for the exposed cohort of patients, and blocks and epidemiologic data are still being collected for the unexposed group. The primary assay to be used on these blocks utilizes the polymerase chain reaction to amplify the gene of interest followed by direct sequencing of the gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-48004-02 EB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Health Effects of Passive Exposure to Cigarette Smoke

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Dale P. Sandler Epidemiologist EB NIEHS

COOPERATING UNITS *(if any)*

The Johns Hopkins University, Training Center for Public Health Research, Hagerstown, MD

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS: 0.25	PROFESSIONAL: 0.25	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A prospective study of health effects of passive smoke exposure has been completed. In this study of over 48,000 adults, the mortality and cancer experience of nonsmokers who lived with smokers in 1963 was compared with that of nonsmokers who did not live with smokers and with smokers. Study subjects were residents of western Maryland who had participated in a special county-wide census in 1963. Data collected at that time on smoking histories for every member of a household were used to derive a measure of exposure to smoke at home for each participant in the study. The cohort was followed through 1975 using medical and vital records. Underlying and contributing causes of death were obtained for census participants who died in the county between 1963 and 1975. Cancer incidence was ascertained through a county-wide cancer register. Results suggest that nonsmokers who live with smokers are at increased risk for death from all causes, at increased risk for death from arteriosclerotic heart disease, and at increased risk for developing certain cancers when compared with nonsmokers who are not exposed to smoke at home. Ongoing analyses using this study include evaluation of the role of cigarette smoke exposure in the etiology of potentially hormonally mediated cancers such as the breast, cervix, prostate and colon. Data have also been used to describe characteristics of never-smokers who have passive smoke exposure at home.

LABORATORY OF BIOCHEMICAL RISK ANALYSIS

LABORATORY OF BIOCHEMICAL RISK ANALYSIS
Summary Statement

The central objective of the Laboratory of Biochemical Risk Analysis (LBRA) is to conduct basic research that will have direct application to various components of the risk assessment process. Our approaches require working knowledge of the emerging concepts in molecular biology, as well as insights into the needs of risk assessors. Consistent with this main objective, LBRA (1) conducts laboratory studies to determine the biochemical mechanisms responsible for epidemiological observations, (2) designs and conducts laboratory studies to enhance the scientific basis for extrapolating risk from animal experiments to human populations, (3) collaborates with epidemiologists and mathematical statisticians to design, implement and interpret research studies involving biochemical applications to risk analysis and biochemical epidemiology, an (4) establishes laboratory research contracts and collaborations outside of DBRA in support of LBRA's overall goals. The organization of LBRA into three sections (Molecular Toxicology, Cellular Epidemiology and Metabolism and Receptors) recognizes our major research directions. Some of the ongoing research projects are summarized in the following paragraphs.

Molecular Toxicology Section

This section conducts studies on (1) chemical-induced DNA damage and cell replication as factors in neoplasia, (2) activation of cancer-causing genes (oncogenes) by carcinogens and its implications for risk estimation, and (3) quantitative relationships between tumor incidence and DNA adducts in rodent model systems as a means of improving methods to estimate human risks from animal studies.

One major focus of these studies is to identify metabolic factors that are involved in cell transformation and progression to neoplasia. This research involves use of both *in vivo* and *in vitro* systems and employ both genotoxic and nongenotoxic compounds. Some of the biological endpoints which are currently being investigated include DNA damage and repair, cytotoxicity, cell turnover, gene expression and the activation of proto-oncogenes in chemical-induced rodent tumors. Results from studies with the nitrosamines 4-(N-methyl-N-nitrosamine)-1-(3-pyridyl)-1-butanone (NNK) and N-nitrosodimethylamine (NDMA) indicate that mouse lung tumors induced by these nitrosamines contain a K-*ras* oncogene which had been activated primarily by a GGT to GAT mutation in the 12th codon. This transition is consistent with the activation of both nitrosamines by formation of the O^6-methylguanine adduct (O^6MG). The potential for using DNA adducts as an index of carcinogenic potential is being evaluated in the Fischer rat. This study will examine the relationship between DNA methylation and tumor induction in target cells over a NNK dose range encompassing 3 orders of magnitude.

The genotoxic potential of methylene chloride, which is weakly mutagenic but induces lung and liver tumors at non-cytotoxic doses in the mouse, was evaluated. Studies indicate that this compound may alter the conformation of the DNA; however at present, stable covalent binding to the DNA has not been demonstrated. Factors involved in promotion and progression to the neoplastic state are also being evaluated by examining the effect of acute and chronic exposure to carcinogens on cell proliferation and gene expression.

The Molecular Toxicology Section has a considerable effort directed towards oncogene activation in chemical-induced and spontaneous rodent tumors as well as some types of human tumors. For example, we have shown K-ras activation as a common event in both human and rodent pulmonary adenocarcinomas. A high percentage (70%) of human lung tumors from smokers were found to contain activated oncogenes and the majority of the detected oncogenes (70%) were detected only by a method seldom used to screen human tumors (nude mouse tumorigenicity assay). Therefore, the actual percentage of human tumors which contain activated oncogenes may be much higher than previously estimated (i.e., approximately 20%). In another study, activated ras-oncogenes were detected in a high percentage of several tumor types induced in rats by benzidine-derived dyes. Human bladder tumors associated with exposure to benzidine, are being examined for the presence of activated ras genes. Oncogene activation is being assessed in spontaneous liver tumors from several mouse strains which are used in carcinogenic studies. Characterization of oncogene activation in human and rodent tumors suggests that activation of a proto-oncogene is a common pathway for tumor induction for some carcinogens. These approaches may help us classify carcinogens according to mechanism of action and may enable us to more accurately estimate risk of cancer in humans exposed to specific classes of carcinogens.

Cellular Epidemiology Section

This section (1) conducts molecular epidemiology studies focussing on markers of exposure and markers of susceptibility, (2) applies ^{32}P-postlabelling methodology to detect DNA adducts in human samples and in animal model systems, and (3) investigates the quantitative relationship between DNA adducts, growth factor pathways and cell transformation in models for mammary carcinogenesis.

The capacity of exogenous compounds or their metabolites to form covalent adducts with DNA is considered a crucial step in the initiation of carcinogenesis. We showed that the DNA of lymphocytes obtained from both nonsmokers and smokers contains multiple adducts, one of which derived from the environmental carcinogen benzo(a)pyrene (BP). These adducts were detected using the ^{32}P-postlabelling method which can detect some DNA adducts at concentrations as low as one adduct in 10^{10} normal nucleotides. However, the level and pattern of adducts obtained varied greatly between individuals and might reflect the genetic variability known to exist in individuals with regard to the activation/deactivation of procarcinogens. In order to understand and predict interindividual variation in adduct formation, we have been studying the role of metabolic activation/deactivation pathways for BP in DNA adduct formation and induction of chromosomal changes following *in vitro* incubation of human lymphocytes with BP. We were also concerned with the question of possible impact of smoking status on these endpoints. Our findings show that there is no smoking-related difference in BP metabolism, DNA adduct formation, SCE induction or ethoxyresorufin-O-deethylase (EROD) activity. We also found that there is a weak correlation between *in vitro* BP metabolism and DNA adduct formation when the whole population is studied. However, if the population is segregated on the basis of glutathione-S-transferase (GST) activity, individuals with high GST activity show a strong correlation between adduct formation and BP metabolism whereas no correlation was seen for persons with low activity. GST is one of the main pathways for deactivation of DNA reactive arene oxides and one form of GST is present in some individuals and absent in others. These studies are providing approaches to develop and evaluate markers of exposure and to identify individuals potentially at risk from exposure to specific chemicals. The major thrust of future studies will be to develop more sensitive markers of chemically-mediated diseases.

There are several factors, such as age of menarche, menopause, and pregnancy, which modify risk for breast carcinoma in human females. However, little or no understanding exists regarding the underlying basic mechanisms or the factors that relate to increased or decreased susceptibility. Estrogenic steroids are important for the development of the mammary glands during puberty and appear to be required for the chemical induction of mammary cancers in rodents and in the growth promotion of human breast cancers. Since epidermal growth factor (EGF) is a potent stimulator of mammary cells _in vitro_, we are investigating EGF mediated proliferative responses to estrogens _in vivo_. The mammary gland is examined under various physiological states, e.g., puberty, pregnancy, and lactation, for expression of EGF-related substances. In other studies, we are examining the relationship of the physiological state of the mammary gland to DNA adduct concentrations following exposure to 7,12-dimethylbenzanthracene (a synthetic mammary carcinogen) over a wide dose range. Our results will be evaluated in relation to the presence of DNA adducts and growth factor constituents in human breast tissue obtained by reduction mammoplasty. These studies should produce a clearer understanding of the mechanisms responsible for the sensitivity of the mammary gland to carcinogens and possibly lead to approaches to minimize risk of mammary cancer.

Metabolism and Receptor Section

This section (1) evaluates metabolic activation/deactivation pathways for chemicals in human samples and animal model systems emphasizing specific cytochrome P-450 isozymes, (2) investigates signal transduction pathways in relation to the tumor promotion process in animal and cell models emphasizing synthetic estrogens and halogenated aromatics as promoters, and (3) conducts pharmacokinetic studies designed to fill knowledge gaps needed for risk assessment of selected chemicals.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) promotes liver tumors in a two-stage model for hepatocarcinogenesis. We have shown that TCDD also increases the metabolism of estrogen in this model to catechol estrogens which are potentially reactive-DNA metabolites. This finding may provide a mechanism for the female specific hepatocarcinogenic actions of TCDD in rats. TCDD alters receptor systems for estrogen, epidermal growth factor (EGF) and glucocorticoids. These receptor systems are involved in cell proliferation pathways and might play a central role in the tumor promotion process. TCDD increases the activity of ethoxyresorufin-O-deethylase, a microsomal enzyme, via the Ah receptor. Dose-response data are presently being generated to determine whether the effects of TCDD on the EGF receptor, estrogen receptor and glucocorticoid receptor are mediated by binding of TCDD to the Ah receptor. We are also determining if human lymphocytes can be classed as responsive or nonresponsive to TCDD by incubating lymphocytes from sensitive and resistant mouse strains, humans and other species with TCDD and evaluating effects on receptor systems. Our results should provide data critical to the risk assessment of dioxin.

The cytochrome P-450 system is the principal monooxygenase system which metabolically activates/deactivates foreign chemicals. Some P-450 enzymes are polymorphic in man and rodents; that is, some individuals are deficient in particular forms of P-450. The human P-450 related to rat P-450g has been reported to be polymorphic in humans (present in 50% of the population and absent in 50%). Our data shows that the rat form is also polymorphic (present or absent in approximately 50% of the population) and may be a good model for the human polymorphism. The principal objective of the present studies are to obtain cDNA probes to further study this defect. A full-length cDNA for rat liver P-450g has been sequenced and a specific oligonucleotide region of the P-450g cDNA was selected. However, analysis of mRNA from +g and -g rats revealed no difference in mRNA content of +g and -g phenotypes suggesting that -g rats might contain a defective mRNA. We are developing oligonucleotide probes to allow us to differentiate between +g and -g phenotypes. We are also in the process of obtaining human liver samples to investigate the role of the human P-450g homolog as a risk factor for liver cancer.

We are studying the pharmacokinetic behavior of benzene and its metabolites as a collaborative project with NTP and the Lovelace Inhalation Toxicology Research Institute. These studies focus on the effect of dose, dose-rate, route of administration (oral or inhalation), species (F344 rat or B6C3F1 mouse) on rates of formation and retention of toxic and nontoxic metabolites in target and nontarget tissues. Oral doses of 50 mg/kg or higher saturated the capacity for benzene metabolism in both rats and mice. The saturating air concentration for benzene metabolism during 6 hr exposures was between 130 and 900 ppm. Mice metabolized more of the benzene by pathways leading to the putative toxic metabolites, benzoquinone and muconaldehyde, than did rats. Our data indicates low affinity, high capacity pathways for detoxification and high affinity, low capacity pathways leading to putative toxic metabolites for benzene. Therefore, if the results of rodent studies performed at high doses were used to assess the health risk at low dose exposures to benzene, the toxicity of benzene might be underestimated. These data have been applied to a physiologically-based pharmacokinetic model for benzene and a risk assessment for benzene using metabolite internal doses and tumor data from the NTP bioassay.

In summary, LBRA has expended considerable effort to develop tractable molecular approaches to (1) understand the underlying mechanisms responsible for epidemiological observations (2) identify populations at risk to the toxic actions of selected chemicals and (3) to remove some of the uncertainty in the risk assessment process. Laboratory scientists are productive, have high visibility and are frequently called upon to present their views on approaches for incorporating molecular studies into risk assessment and epidemiology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21024-07 LBRA

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Drug Metabolizing Enzymes in Animal Models and Human Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Joyce Goldstein	Pharmacologist	LBRA	NIEHS
Others:	H. Yeowell	Visiting Associate	LBRA	NIEHS
	P. McClellan-Green	Biologist	LBRA	NIEHS
	P. Linko	Chemist	LBRA	NIEHS
	G. Lucier	Research Chemist	LBRA	NIEHS

COOPERATING UNITS *(if any)*

M. Negishi, Laboratory of Pharmacology, DIR
D. Waxman, Harvard University

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Metabolism and Receptors Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	1.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The cytochrome P-450 system is the principal monooxygenase system which metabolizes foreign chemicals to both inactive compounds as well as activating them to mutagens and carcinogens. Some of these enzymes are polymorphic in both man and rodetns. A polymorphism in a human P-450 (Hpl-1) immunochemically related to rat liver P-450g has recently been reported. This P-450 appears to be present in about 50% of the human population and absent in the remainder. The rat appears to be a good model for the human since rat P-450g is also present in 50% of the population and absent in the remainder. In order to obtain molecular probes to further study this defect in ras and humans, a cDNA library was made from a high P-450g rat. Several cDNAs were identified with specific antibody to P-450g. A full-length cDNA for P-450g has been completely sequenced. This cytochrome contains considerable homology to other cytochromes in the P-450IIc subfamily and to the cDNA for the human P-450, Hpl-1. Based on computer analysis, a specific oligonucleotide region of the P-450g cDNA was selected for use in Northerns and Southerns. This oligoprobe confirmed the P-450g in polymorphism rats. However, Northern analysis of RNA from +g and -g rats revealed no difference in mRNA content of +g and -g phenotypes suggesting that -g rats might contain an alternate, perhaps defective mRNA. Additional studies underway include development in the fetus and neonate and studies to determine whether any extraheptic tissues (more readily studied from humans) contain this RNA. A cDNA library ahs been constructed from -g male rats, and several putative clones have been selected by hybridization studies. These clones are presently being sequenced to determine whether they are defective, and to develop oligonucleotide probes to allow us to differentiate between +g and -g phenotypes. We are in the process of obtaining human liver samples to investigate the possible role of the human P-450g homolog as a risk factor for liver cancer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-35005-09 LBRA

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Carcinogen-Induced DNA Damage and Cell Transformation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Marshall Anderson	Research Chemist	LBRA	NIEHS
	Steven Belinsky	Senior Staff Fellow	LBRA	NIEHS
Others:	C. White	Biologist	LBRA	NIEHS
	T. Devereux	Biologist	LBRA	NIEHS

COOPERATING UNITS *(if any)*

Dr. Robert Maronpot, Chemical Pathology Branch, NIEHS
Dr. Stephen Hecht, American Health Foundation

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Molecular Toxicology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.2	1.2	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major focus of these studies is to identify metabolic factors that are involved in cell transformation and progression to neoplasia. These research goals involve use of both *in vivo* and *in vitro* systems and employ both genotoxic and nongenotoxic compounds. Some of the biological endpoints which are currently being investigated include DNA damage and repair, cytotoxicity, cell turnover, gene expression and the activation of proto-oncogenes in chemical-induced rodent tumors. Results from studies with the nitrosamines 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N-nitrosodimethylamine (NDMA) indicate that mouse lung tumors induced by these nitrosamines contain a K-*ras* oncogene which had been activated primarily by a GGT to GAT mutation in the 12th codon. This GC to AT transition is consistent with the activation of both nitrosamines by formation of the O^6-methylguanine adduct (O^6MG). The potential for using DNA adducts as an index of carcinogenic potential is being evaluated in a dose response carcinogenicity study with NNK in the Fischer rat which will examine the relationship between DNA methylation and tumor induction in target cells over a dose range encompassing 3 orders of magnitude. The further importance of the O^6MG adduct as a carcinogenic lesion was demonstrated in studies which evaluated the repair of this adduct in lung cells. The Clara cell, which was found in previous studies to accumulate high concentrations of the O^6MG adduct, appears virtually deficient in the ability to remove this adduct, another factor which may contribute to cell initiation by alkylating agents in the lung. The genotoxic potential of methylene chloride, which is weakly mutagenic, but induces lung and liver tumors at non-cytotoxic doses in the mouse was evaluated. Studies indicate that this compound may alter the conformation of the DNA; however at present, stable covalent binding to the DNA has not been demonstrated. Factors involved in promotion and progression to the neoplastic state are also being evaluated by examining the effect of acute and chronic exposure to carcinogens on cell proliferation and gene expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-43009-05 LBRA

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Kidney and Nutritional Factors in Metabolism of Toxic and Essential Metals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Suzanne Snedeker	Senior Staff Fellow	LBRA	NIEHS
Others:	Chris Miller	Biologist	LBRA	NIEHS
	Rong-fang Hu	Visiting Fellow	LBRA	NIEHS
	George Lucier	Chief	LBRA	NIEHS
	D. Dixon	Expert	CPB	NIEHS
	R. Maronpot	Head, Exp. Path.	CPB	NIEHS

COOPERATING UNITS *(if any)*

Dr. G. A. Johnson, Director of Diagnostic Physics, Duke Medical Center

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0 5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Increasing evidence indicates that the carcinogenicity of a metal depends on its "cellular bioavailability"; its ability to enter and accumulate in target cells and interact with genetic material. Iron has been implicated in the generation of highly reactive hydroxyl radicals, which can cause damage to DNA directly. The ferric form of the metal chelator ferric-nitrilotriacetate (FeNTA) has been reported to induce kidney tumors, potentiate an excessive excretion of essential urinary metals and cause a diabetic-like iron overload syndrome similar to that seen in human idiopathic hemochromatosis. We have used FeNTA to study mechanisms of metal-induced cancerous and precancerous lesions using a rat model. Male rats received daily injections of ferric-nitrilotriacetate (FeNTA) for 9 weeks; control animals received equivalent amounts of nitrilotriacetate, ferric nitrate, iron dextran or saline. Protein and glucose were detected in the urine of FeNTA animals beginning at 4 weeks and persisted until the injections were terminated. A protein detected in the urine of FeNTA animals by polyacrylamide electrophoresis was determined to be the iron binding protein, transferrin by Western blot analysis. The excessive excretion of zinc, copper and iron in the FeNTA treated animals returned to baseline levels within 2 to 3 weeks after terminating the injections. FeNTA induced tubular renal adenocarcinomas in 60% of the surviving animals; no tumors were detected in controls. Preneoplastic lesions in the livers of several FeNTA animals were detected by gamma-glutamyl transpeptidase positive foci. Tissue distribution of iron, tissue metallothionein, and the activity of radical scavenging enzymes will be determined, since metalloproteins and antioxidant enzymes may affect metal induced free radical damage to DNA. A subset of the FeNTA dosed animals have been used to develop and refine magnetic resonance imaging (MRI) techniques to monitor kidney tumor growth over extended periods of time in the live animal.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-46003-04 LBRA

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Lymphocyte Markers for Evaluating Exposure and Biologically Effective Dose

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Claudia Thompson	Senior Staff Fellow	LBRA	NIEHS
	George Lucier	Chief	LBRA	NIEHS
Others:	J. Lambert	Bio. Lab. Tech.	LBRA	NIEHS
	D. DiAugustine	Research Chemist	LBRA	NIEHS
	G. Jahnke	IRTA	LBRA	NIEHS
	Y. Liu	Visiting Fellow	LBRA	NIEHS
	Z. McCoy	Bio. Lab. Tech.	LBRA	NIEHS
	J. Goldring	Guest Researcher	LBRA	NIEHS

COOPERATING UNITS *(if any)*
Epidemiology Branch, DBRA

LAB/BRANCH
Laboratory of Biochemical Risk Analysis

SECTION
Cellular Epidemiology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	2.2	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The long range objective is to evaluate the possible use of human lymphocytes as potential markers of human exposure and susceptibility. The relationship between chemical exposure and changes in biochemical or cytogenetic parameters will be investigated in both defined human populations and animal models. The effect of pharmacokinetic parameters of activation/deactivation on the generation of biologically effective doses (i.e., DNA adducts) and the resulting consequences on biological endpoints such as SCEs and DNA damage will be evaluated in human lymphocytes following _in vitro_ exposure to chemicals. A study was undertaken to (1) determine the role of metabolic activation/deactivation pathways on DNA adduct formation and SCE induction by incubating lymphocytes _in vitro_ with benzo(a)pyrene (BP) and (2) determine whether smoking exposure resulted in smoking-related adducts which could be measured in lymphocytes by ^{32}P-postlabelling methods. Endpoints studied included BP-DNA adduct formation, BP metabolism, ethoxyresorufin-O-deethylase (EROD) activity, glutathione-S-transferase (G-ST) activity and SCE induction. We showed that _in vitro_ BP metabolism, EROD activity, SCE induction by BP or BPDE-dG adduct levels did not differ between smokers and nonsmokers. However, we did observe a large inter-individual variation for these endpoints. We found that the correlation between lymphocyte BP metabolism and DNA adduct formation was weak for the whole population, however when the population was segregated on the basis of G-ST activity (deactivation pathway for reactive epoxides) there was a very strong correlation between BP metabolism and DNA adduct formation for persons with high G-ST activity. Individuals with low activity showed a very weak correlation. ^{32}P-postlabelling of human lymphocytes resulted in the detection of several DNA-adduct spots which varied considerably among individuals. However none appeared to be smoking related. Future studies will determine the role of G-ST activity and whether there is a correlation between endogenous adduct levels and a persons capacity to metabolize chemicals to DNA reactive species following _in vitro_ exposure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-46004-04 LBRA

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Receptor Interactions and Liver Tumor Promotion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	George Lucier	Chief	LBRA	NIEHS
	Joyce Goldstein	Pharmacologist	LBRA	NIEHS
Others:	F.-H. Lin	IRTA	LBRA	NIEHS
	G. Clark	IRTA	LBRA	NIEHS
	P. Linko	Chemist	LBRA	NIEHS
	T. Sloop	Biologist	LBRA	NIEHS

COOPERATING UNITS *(if any)*

University of North Carolina
Laboratory of Pharmacology, NIEHS
Statistics and Biomathematics Branch, NIEHS

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Metabolism and Receptors Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The long-range plan of this project is to evaluate the actions of receptors for halogenated aromatic chemicals in relation to the carcinogenic potency of these compounds. These studies focus on receptor mediated effects in short-term models, in vitro models, and in the two-stage model for promotion of hepatocarcinogenesis. One goal of this project is to evaluate the role of the Ah receptor in the action of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on other receptor systems such as the glucocorticoid receptor, epidermal growth factor (EGF) receptor, and the estrogen receptor. These receptors are known to modulate cell proliferation pathways and ongoing studies are comparing the dose-response for the effects of TCDD on these receptors in livers of wild type (Ah+) and congenic strains of mice containing a defective Ah receptor (Ah-). If the effects of TCDD are mediated by the Ah receptor, a 10-fold difference in the dose response is predicted between Ah- and Ah+ mice. Studies will also examine effects on protein kinases and phosphorylation (signal transduction pathways) of the receptor proteins. TCDD has been shown to promote the number of enzyme-altered foci in DEN initiated intact female rats and to increase metabolic activation of estrogen in this model. Additional studies will compare the effects of TCDD on tumors, enzyme altered foci and receptor systems in intact and ovariectomized rats using this model to determine the possible role of estrogens and DNA adducts in this process. We will also attempt to develop in vitro models to obtain additional information on the mode of action of TCDD and obtain data on structure-activity relationships. These will include wild type and receptor defective hepa 1c1c7 cells. Additional in vitro systems used for dose-response analysis will include lymphocytes from humans as well as Ah- and Ah+ animals to determine whether humans more nearly resemble responsive or nonresponsive strains. Data generated from this project should provide information useful in the risk assessment of the ubiquitous halogenated aromatics.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-ES-46005-04 LBRA

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Oncogene Activation and Expression in Rodent Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Steve Reynolds	Senior Staff Fellow	LBRA	NIEHS
	Marshall Anderson	Research Chemist	LBRA	NIEHS
Others:	Colleen Hunnicutt	Biologist	LBRA	NIEHS
	Rachel Patterson	Microbiologist	LBRA	NIEHS
	Vicki Burnett	Biologist	LBRA	NIEHS
	Katie Brown	Biologist	LBRA	NIEHS
	Urs Candrian	Visiting Fellow	LBRA	NIEHS
	Fred Tyson	Staff Fellow	LBRA	NIEHS
	Bengt Widegren	Visiting Fellow	LBRA	NIEHS

COOPERATING UNITS *(if any)*

Dr. Robert Maronpot, National Toxicology Program, NIEHS

LAB/BRANCH
Laboratory of Biochemical Risk Analysis

SECTION
Molecular Toxicology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.5	4.0	3.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recent work from two independent lines of investigation has merged to suggest that neoplasia results, at least in part, from the abnormal activation of a relatively small number of cellular genes. These genes, termed proto-oncogenes, can be activated by genetic alterations which range from point mutations to gross DNA rearrangements such as translocation or amplification. Induction of tumors in rodents by genotoxic carcinogens results in activation of specific oncogenes with high frequency. We have investigated oncogene activation in chemical-induced and spontaneous rodent tumors as well as some types of human tumors. For example, we have shown K-ras activation as a common event in both human and rodent pulmonary adenocarcinomas. In addition, several non-ras genes have been detected in human lung tumors. A high percentage (70%) of human lung tumors from smokers were found to contain activated oncogenes and the majority of the detected oncogenes (70%) were detected only by a method seldom used to screen human tumors (nude mouse tumorigenicity assay). Therefore, the actual percentage of human tumors which contain activated oncogenes may be much higher than previously estimated (i.e., approximately 20%). In another study, activated ras-oncogenes were detected in a high percentage of several tumor types induced in rats by benzidine-derived dyes. Human bladder tumors associated with exposure to benzidine are being examined for the presence of activated ras genes. Oncogene activation is being assessed in spontaneous liver tumors from several mouse strains which are used in carcinogenic studies. Characterization of oncogene activation in human and rodent tumors suggests that activation of a proto-oncogene is a common pathway for tumor induction for some carcinogens. These approaches may enable us to more accurately estimate risk of cancer in humans exposed to specific classes of carcinogens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-48005-01 LBRA

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical Mechanisms Related to Risk Factors of Mammary Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Richard DiAugustine	Research Chemist	LBRA	NIEHS
Others:	S. Snedeker	Senior Staff Fellow	LBRA	NIEHS
	G. Jahnke	IRTA	LBRA	NIEHS
	C. Brown	Biologist	LBRA	NIEHS
	C. Miller	Biologist	LBRA	NIEHS
	G. Lucier	Chief	LBRA	NIEHS

COOPERATING UNITS *(if any)*

Epidemiology Branch, DBRA
University of North Carolina, Chapel Hill, NC

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Cellular Epidemiology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	1.5	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Since this is a new project any synopsis will consist for the most part, of proposed experiments. The relatively high incidence of carcinoma of the breast among women in the United States has prompted the need to understand the biochemical basis for susceptibility of this disease. Ovarian estrogens, which are considered essential for the growth of mammary ducts during puberty, apparently have a critical role as growth promoters in mammary cancer. In one study we are investigating the potential for local growth factor pathways to mediate estrogen-stimulated mouse mammary cell growth. We are specifically investigating the potential role of polypeptides that activate the epidermal growth factor (EGF) receptor, since previous studies have shown that isolated mammary epithelial cells are highly sensitive in vitro to the mitogenic actions of EGF. Efforts to immunolocalize EGF or the EGF precursor in mammary cells of young CD-1 mice have yielded negative results so far and in vivo pellets containing anti-EGF IgG have failed to inhibit elongation of the ducts in young mice. We are also investigating the potential for -transforming growth factor (TGF) to function as an autocrine factor since this polypeptide also has EGF-receptor activity. In other studies, we found that prepro EGF mRNA (4.7 kilobases) was elevated in the mouse lactating mammary gland. Since the lactating gland exhibits negligible cell proliferation, we propose that the EGF precursor is processed to elaborate EGF at the cell surface as a constituent of milk. In another study, we are examining the permanent influence that early full-term pregnancy exerts on the capacity of the mouse mammary gland to repair DNA adducts following treatment with 7,12-dimethylbenzanthracene (DMBA), a mammary procarcinogen. It is well known that early full-term pregnancy is a negative risk factor in human breast cancer. We plan to utilize the ^{32}P-postlabeling method to monitor the type and level of adduct formed in vivo in nonparous and parous animals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-70069-06 LBRA

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

DNA Adducts in Human Lymphocytes and Hormone-Dependent Cancers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Richard DiAugustine	Research Chemist	LBRA	NIEHS
	George Lucier	Chief	LBRA	NIEHS
Others:	C. Thompson	Senior Staff Fellow	LBRA	NIEHS
	G. Jahnke	IRTA	LBRA	NIEHS
	M. Walker	Chemist	LBRA	NIEHS

COOPERATING UNITS *(if any)*

Epidemiology Branch, DBRA
University of North Carolina, Chapel Hill, NC
Genetic Toxicology Division, Environmental Protection Agency

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Cellular Epidemiology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It is well known that chemical carcinogens have the capacity to form covalent DNA adducts directly or indirectly through their metabolites. The formation of these adducts is considered to be the common mechanism by which structurally diverse chemicals ultimately produce mutations and cancer. Identification of DNA adducts might provide a way to monitor human exposure to potential genotoxic agents. We had shown earlier that human lymphocytes incubated with 10^{-6} M benzo(a)pyrene, an environmental procarcinogen, yield only one major adduct, both by high performance liquid chromatography and ^{32}P-postlabeling analysis. Marked variation (>10 fold) in the level of adduct formation was observed in lymphocytes from different donors; no significant difference in adduct formation in vitro was observed between smokers and nonsmokers. These findings suggest that lymphocytes can be used to assess the capacity of compounds to react covalently with DNA in human cells. We also detected DNA adducts in lymphocytes analyzed directly by the 32P-postlabeling method that was modified to give enhanced sensitivity by treatment of nucleotides with nuclease P1. Thin layer chromatography maps revealed multiple adducts at variable levels in all specimens, including those from nonsmokers; adduct occurrence was approximately 1 per 10^7 - 10^8 nucleotides. One of the adducts co-migrated with the major adduct obtained in benzo(a)pyrene-treated lymphocytes. The results suggest that aromatic hydrocarbon-related adducts accumulate in nonsmokers as well as smokers and that the profile and extent of these adducts can vary greatly among individuals. This study is being extended to include the detection of DNA adducts in human breast tissue. Although estrogens can promote growth through the mammary gland, perhaps through a growth factor pathway, the etiologic factors that make this organ, or other estrogen-responsive organs, susceptible to cancer are not known. The DNA adduct level and pattern of breast tissue obtained by reduction mammoplasty will be correlated with patient profile factors related to known risk factors for breast cancer.

STATISTICS AND BIOMATHEMATICS BRANCH

STATISTICS AND BIOMATHEMATICS BRANCH
Summary Statement

The Statistics and Biomathematics Branch (SBB) of the Division of Biometry and Risk Assessment (DBRA) carries out basic and applied research dealing with statistical aspects of environmental health issues, and entails both the application of existing techniques and the development of new statistical methodology as the need arises. This research often involves collaboration with other investigators from the Division of Toxicology Research and Testing (DTRT), the Division of Intramural Research, and DBRA's Epidemiology Branch and Laboratory of Biochemical Risk Analysis.

Organizationally, the Branch is divided into two sections: the Statistics Section and the Biomathematics Section. The primary responsibilities of the Statistics Section are to carry out methodological research on toxicological and epidemiological problems arising within the Institute's various research programs and to provide statistical and computational support to Institute scientists.

The large database of carcinogenicity data generated by the DTRT has been used to address important issues such as developing statistical methodology for the analysis of tumor data, evaluating the relationship between toxicity, genotoxicity, and carcinogenicity in laboratory rodents, and identifying and controlling for potential sources of variability in tumor incidence. The results of these three research efforts are summarized briefly below.

Statistical procedures were developed for evaluating tumor data that do not require cause of death information or numerous interim kills. Particularly promising is a method of analysis that weights the contribution of animals that die tumor-free based upon a hypothesized form of the tumor onset distribution. The resulting procedure is a simple modification of the Cochran-Armitage trend test and may ultimately result in an important advance in the statistical evaluation of tumor data.

An evaluation of the relationship between toxicity, genotoxicity, and carcinogenicity in laboratory rodents indicated that only a relatively small proportion (10-15%) of positive carcinogenicity studies exhibited the types of target organ toxicity that could have been the cause of all observed carcinogenic effects. Furthermore, no apparent difference in mutagenicity was observed between "high dose only" carcinogens and the entire set of carcinogens.

An examination of tumor data from NTP laboratory animal carcinogenicity studies indicated that inter-laboratory variability and time-related trends were significant sources of variability. Ongoing research includes a re-evalation of tumor diagnoses from early NCI and current NTP studies to identify the specific factors responsible for this variability.

Future research will continue to focus on these and other related statistical issues in the design, analysis, and interpretation of long-term animal studies, such as control of potentially confounding fa ctors, survivaladjusted methods of analysis of tumor data, and use of historical control information. These efforts are essential to increase our understanding of the various factors that influence the results of carcinogenicity testing in laboratory animals and to enhance the utility of these studies in the overall assessment of human health risk.

The Statistics Section research efforts in the area of epidemiological or human studies is directed toward gaining additional insight into the uses and limitations of existing study designs and methodologies, and investigating the adaptation or development of new statistical methods when such a need is indicated. During the past year, work has concentrated in two main areas: statistical analysis of spatial and temporal clustering to investigate the etiology of disease and mathematical modeling of various aspects of reproduction. Specific research accomplishments include the development of a new statistic for detecting seasonality of events (such as disease onset), the development of an improved algorithm for identifying the time of ovulation using estrogen and progesterone levels, and the development of proportional hazard type models for fertility based on the number of menstrual cycles required to achieve pregnancy. Efforts are underway to develop models for estimating the probability of conception on different days of the menstrual cycle. Such models will potentially enhance our ability to detect deleterious effects of environmental agents on fertility.

In addition to its research activities, the Statistics Section provides computational and statistical support for various research projects within the Institute. These activities range from routine data analysis to more extensive collaborative efforts that result in joint publications in subject matter journals.

The main goal of the Biomathematics Section is to explore the utilization of mathematical theory in addressing problems arising in the biological sciences, particularly in the modeling of biological processes. Much of the current research effort has been motivated by problems in theoretical population genetics, genetic toxicology and risk assessment.

The advent of restriction enzyme analysis, gene cloning and DNA sequencing techniques has brought about a rapid growth of knowledge about the structure of the genetic material of a cell. Applications of these techniques have led to an accumulation of data on DNA sequences. An important use of these data is to address the fundamental questions of how much genetic variability exists in populations and how this variability is generated and maintained. The statistical methods used to analyze these data are based on population genetic models and so it is essential that these models incorporate the concepts of mutation, selection and recombination. The coalescent process, which describes the ancestral relationships for a random sample of genes, has been particularly useful for analyzing DNA sequence data, assuming selective neutrality. Current research has focused on studying the coalescent process for a selected locus and a neutral locus linked to a selected locus. A major result of this research is a new statistical method for assessing the presence of selection. Future research in this area will study the structure of the coalescent process for geographically subdivided populations and populations undergoing periodic selection.

Important scientific goals in genetic toxicology are to identify genotoxic agents and understand their possible mechanisms of action. To help in accomplishing these goals, it is critical to have appropriate statistical methods for analyzing genotoxicity data from a variety of animal and microbial systems. Current research has focused on the development of nonparametric statistical analyses and designs for experiments assessing chromosomal loss in yeast. The dose-response data for these experiments often exhibit a unimodal "umbrella" pattern and so the statistical analyses were designed to allow for nonmonotone curvature. The methods developed can be applied to any dose-response data that exhibit a downturn. Future research is planned to investigate other forms of nonparametric tests and more complex response models.

Finally, the Biomathematics Section develops methods for estimating the risk associated with low levels of exposure to hazardous environmental agents. The key problems are how to extrapolate high dose results in laboratory animals to low dose exposures and how to extrapolate low dose risk estimates in animals to humans. Within species extrapolation is typically based on an underlying mathematical model which is used to estimate the animal's risk at low exposure doses. An important research objective in this area is to improve the validity of extrapolation models by incorporating as much of the current knowledge about the underlying biological processes as possible. Current research has investigated the statistical properties of a recently proposed two-stage clonal model for initiation and promotion. Of particular interest is the quantifying of the variability of risk estimates when data from related experiments are used to estimate some of the model's parameters. Species to species extrapolation is typically accomplished using scaling factors to estimate interspecies equivalency. Physiologically based pharmacokinetic models have recently been proposed as a more biologically relevant way to perform species to species extrapolation. Research in this area has focused on quantifying the variability of risk estimates obtained in this way. The results show that these estimates are more variable than traditional estimates and that the magnitude of the variability depends upon how much variation is associated with the data that is used to estimate the parameters of the pharmacokinetic model. Future research will continue to focus on mechanistic models of carcinogenesis and the use of physiological based pharmacokinetic models in risk estimation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 40004-11 SBB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Statistical Methods in Epidemiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Beth Gladen	Statistician	SBB	NIEHS
OTHERS:	Clarice Weinberg	Mathematical Statistician	SBB	NIEHS
	Allen Wilcox	Medical Officer	EB	NIEHS

COOPERATING UNITS *(If any)*

Epidemiology Branch, NIEHS
Biostatistics Department, Columbia University

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

Statistics Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project encompasses the development and evaluation of statistical methods appropriate for epidemiologic research. This year work has concentrated in two main areas. The first is statistical analysis of spatial and temporal clustering to investigate the etiology of disease, including the possible roles of environmental factors. The second is mathematical modelling of various aspects of reproduction. Of particular interest are models which can explicitly incorporate the effects of environmental insults. A list of specific developments follows. (1) A new measure of geographic clustering was proposed. (2) A new statistic for detecting seasonality of events (such as disease onset) was developed and characterized. This statistic is designed to be particularly sensitive to a pattern where three seasons have similar incidence but one has an increased incidence. (3) Proportional hazards type models for fertility were developed, based on the number of menstrual cycles required to achieve pregnancy. Using such models, we can assess the aggregated effects on fertility (and preclincial loss) of covariates including environmental agents. (4) An improved algorithm for identifying the time of ovulation using estrogen and progesterone levels was developed; this is a useful prerequisite for various other analyses. (5) A mathematical model has been proposed which can usefully be applied to data on early pregnancy loss in women undergoing in vitro fertilization/embryo transfer. This model exploits the fact that several embryos are transferred, in order to separate effects on the maternal uterus from effects on the zygotes. (6) In ongoing work, we are attempting to develop models for conception following intercourse on different days of the menstrual cycle. Currently, we are focusing on very simple models; potentially effects of environmental exposures on ovum viability and sperm lifetime could be estimated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 45001-08 SBB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Experimental Design and Data Analysis Methodology for Animal Experiments

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Joseph K. Haseman	Research Mathematical Statistician	SBB	NIEHS
OTHERS:	Christopher J. Portier	Mathematical Statistician	SBB	NIEHS
	Gregg E. Dinse	Mathematical Statistician	SBB	NIEHS
	A. John Bailer	Staff Fellow	SBB	NIEHS

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

Statistics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	2.7	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is concerned with statistical methodology issues involved in the design, analysis, and interpretation of laboratory animal experiments, with particular emphasis on two-year carcinogenicity studies. Experimental design issues include examining potential sources of variability in tumor incidence. Inter-laboratory differences and time-related trends were found to be significant sources of variability. Future research plans include a re-evaluation of tumor diagnoses from early NCI and current NTP studies, to identify the specific factors responsible for this variability. Research in the area of data analysis has focused on the development of statistical procedures for evaluating tumor data that do not require cause of death information or numerous interim kills. Particularly promising is a procedure that weights the contribution of animals that die tumor-free based upon a hypothesized form of the tumor onset distribution. The resulting procedure is a simple modification of the Cochran-Armitage trend test and may ultimately result in an important advance in the statistical evaluation of tumor data. An evaluation of the relationship between toxicity, genotoxicity, and carcinogenicity in laboratory rodents indicated that only a relatively small proportion (10-15%) of positive carcinogenicity studies exhibited the types of target organ toxicity that could have been the cause of all observed carcinogenic effects. Furthermore, no apparent difference in mutagenicity was observed between "high dose only" carcinogens and the entire set of carcinogens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 48002-01 SBB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Statistical Models in Toxicology and Biochemistry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Christopher J. Portier	Mathematical Statistician	SBB	NIEHS
OTHERS:	Norman L. Kaplan	Mathematician	SBB	NIEHS
	Annette Kopp	Visiting Fellow	SBB	NIEHS
	A. John Bailer	Staff Fellow	SBB	NIEHS
	George Lucier	Section Chief	LBRA	NIEHS

COOPERATING UNITS *(if any)*

Laboratory of Biochemical Risk Assessment, DBRA

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

Biomathematics Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mathematical and statistical models are important tools in the design, analysis and interpretation of experiments in toxicology and biochemistry. However, many of the properties of the existing models are poorly understood. In addition, many of the existing models do not adequately describe current research findings in these areas. The goal of this project is to increase our understanding of the use and application of existing models in toxicology and biochemistry and to develop and implement new models to aid in explaining new research findings. One research effort concerns the use of physiologically based pharmacokinetic models in estimating carcinogenic risk from exposure to chemicals. A method was developed for incorporating all the sources of variability into the risk estimation procedure to arrive at an estimate of the overall variability. It was shown that the use of these models was likely to increase the variability of the risk estimates. In another study, the ability of carcinogenicity data to estimate parameters in a clonal two-stage model of carcinogenesis was examined. The findings indicated that the carcinogenicity data was inadequate for this purpose and additional information must be used. Research on this topic is continuing with a focus on redesigning carcinogenicity experiments to provide better information on these model parameters. Other research topics concern correcting for survival differences in animal carcinogenicity data and the potential risks from using inappropriate models in estimating risks of developmental defects from exposure to chemicals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 44002-12 SBB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mathematical Modeling of Molecular Phenomena

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Norman L. Kaplan	Research Mathematician	SBB	NIEHS
OTHERS:	Charles H. Langley	Research Chemist	LG	NIEHS
	Richard R. Hudson	Staff Fellow	SBB	NIEHS

COOPERATING UNITS *(if any)*

Laboratory of Animal Genetics, LRDT

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

Biomathematics Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	2.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In view of the recent explosive accumulation of data on DNA sequences, it is important for the analysis of these data to have adequate mathematical models and statistical methods. During the past year work has continued on studying the properties of the coalescent process for samples of genes from populations undergoing selection. Four topics were investigated. (1) The structure of the coalescent process for a neutral locus linked to a selected locus was determined. This work was needed to determine the analytic properties of a new statistical method for identifying the presence of selection. (2) To obtain numerical results for the coalescent process for selective models often requires solving a system of nonstandard singular second order differential equations. Since standard software packages do not succeed here, it was necessary to develop a numerical recipe especially suited to this system. (3) The coalescent process was used to study the evolutionary effects of periodic selection. This work showed that the pertinent parameter is r/s where r is the rate of recombination and s is the strength of selection. (4) The final area deals with the coalescent process for subdivided populations, in particular populations that are undergoing selection. This work is relevant to the analysis of recent Adh data for D. melanogaster.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 48001-01 SBB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Statistical Analysis of Data from Genotoxicity Experiments

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Walter W. Piegorsch	Mathematical Statistician	SBB	NIEHS
OTHERS:	Michael A. Resnick	Supervisory Research Geneticist	CGTB	NIEHS
	Errol Zeiger	Supervisory Microbiologist	CGTB	NIEHS

COOPERATING UNITS *(if any)*

Cellular Genetics and Toxicology Branch, DTRT

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

Biomathematics Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A genotoxin is an agent that is capable of inducing damage to or heritable changes in genetic material. Such agents are thought to play a significant role in carcinogenesis, and possibly aging and heart disease as well. Scientific research in genetic toxicology aims to identify and understand genotoxins and their possible mechanisms of action. The primary focus of this project is the development of appropriate statistical methodologies for analysis of genotoxicity data in a variety of animal and microbial systems. Investigations were undertaken to develop statistical analyses and designs for experiments assessing chromosome loss in yeast. Data from these biological assays often exhibit a unimodal "umbrella" pattern in the dose-response, and the statistical analyses were designed to take this possible nonmonotone curvature into account. Minimal design configurations were identified, below which the test procedures lose power to detect true "umbrella" departures from a no-effect model. These methods are applicable to any toxicological assay where downturns in dose-response are evidenced. Additional research examined quantitative trends in the association between mutagenic and carcinogenic measures of potency. A significant positive correlation was seen between the two potencies, although the spread of the data cloud was too great for the overall result to be of predictive use. Classification by chemical class further indicated strong positive correlations for nitrosamines and related compounds. These compounds are known to possess strong carcinogenic potential.

DIVISION OF TOXICOLOGY RESEARCH AND TESTING

DIVISION OF TOXICOLOGY RESEARCH AND TESTING
Summary Statement

The Division of Toxicology Research and Testing (DTRT), develops scientific information about potentially toxic and hazardous chemicals. The DTRT, within the National Institute of Environmental Health Sciences (NIEHS), is the largest component of the National Toxicology Program (NTP) and the NTP is also based at NIEHS. The other contributing components of the NTP are the National Center for Toxicologic Research of the Food and Drug Administration and the National Institute for Occupational Safety and Health of the Centers for Disease Control.

The DTRT devotes its primary efforts to basic and applied toxicology research; to comprehensive assessment of the toxic potential of chemicals; and to the development, validation, and evaluation of toxicology methods and assays. Relevant toxicological findings and evaluations are provided to the U.S. Government's research and regulatory agencies, to the national and international scientific communities, and to the public. The DTRT concentrates its major efforts in three interactive areas: (1) expanding toxicological data of chemicals nominated, selected, and studied in the NTP; (2) developing, evaluating, and validating a series of experimental designs, protocols, and biologic assays appropriate for research and regulatory needs; and (3) collecting, evaluating, sharing, and disseminating toxicological information.

The toxicology advances, results, and interpretative information generated by the DTRT and NTP are used to protect the health of the American people and in the primary prevention of chemically induced diseases.

To accomplish its major goals, the DTRT program segments are grouped into discipline-oriented, yet fully coordinated, branches: Carcinogenesis and Toxicologic Evaluation (Dr. J. Selkirk), Cellular and Genetic Toxicology (Dr. R. Tennant), Chemical Pathology (Dr. G. Boorman), Systemic Toxicology (Dr. B. Schwetz), and Data Management and Analysis (Dr. D. Hoel; part of the Division of Biometry and Risk Assessment). Dr. Richard A. Griesemer, formerly Director, Biology Division, Oak Ridge National Laboratory, joined the Division as Director, DTRT, in July.

In addition to basic and applied toxicologic research activities, the functions of the DTRT branches include scientific and technical oversight and monitoring of NTP collaborating and contract laboratories throughout the U.S.

Highlights of DTRT/NTP activities:

- o Fifteen draft NTP Technical Reports on toxicology and carcinogenesis studies were peer reviewed in the two public sessions of the Board of Scientific Counselors' Peer Review Panel in FY 1988. Those chemicals exhibiting clear evidence of carcinogenicity in rats or mice in these studies included dichlorvos (an insecticide), d-limonene (a natural ingredient of citrus), nitrofurantoin (an antibacterial), ochratoxin A (a fungal toxin), pentachlorophenol (a wood preservative), and tribromomethane (a solvent and drinking water contaminant). Those showing no evidence of carcinogenicity included benzyl alcohol (a textile dye additive), 2,4-dichlorophenol (an intermediate in herbicide production), and tetracycline hydrochloride (an antibiotic). Six other chemicals showed limited or equivocal evidence of carcinogenicity.

- During fiscal year 1988, pathology data from 40 studies (one study equals one animal species per chemical and includes approximately 40 tissues, blocks, and slides per animal, or about 60,000 specimens) submitted to the NTP by contract laboratories were reviewed and evaluated. Histopathology quality assessments were completed on 41 studies, and pathology reviews were conducted on 33 two-year or lifetime studies. In addition, 48 prechronic toxicity studies were also evaluated. These data, findings, and conclusions go into technical reports and are made available to nearly 1,500 individuals and institutes on a regular mailing list, and to anyone upon request.

- The Comprehensive Quality Assurance Audit of Gulf South Research Institute (GSRI) studies has been completed. Findings from the review of the archival records for each study are presented in separate audit reports, while general information and important findings that cut across two or more of the GSRI studies are summarized in a General Information Report. Because of concerns about the quality of the studies as compared to current standards, NTP management has decided that none of the GSRI studies will be printed in the NTP Technical Reports series. Instead, select pathology data from these studies will be available, along with audit findings, in a series of chemical-specific data collections.

- The DTRT study on the health effects of groundwater contaminants is continuing. Extensive analytical chemistry on the stability of the 25-chemical mixture (19 organic and 6 inorganics) in drinking water under experimental conditions was carried out. The battery of toxicology studies consists of: (1) 6-month toxicity studies in rats and mice including palatability and dose-range finding studies; (2) short-term (14-day and 13 week) immunotoxicity in female mice, developmental toxicity in pregnant rats, and a 13-week male reproductive toxicity study in mice; (3) biochemical studies in vivo and in vitro on hepatic mitochondrial respiration and enzymatic activities in male rats; (4) myelotoxicity studies in mice; (5) continuous breeding reproductive toxicity studies in mice and rats; and (6) in vivo cytogenetic studies in rats and mice.

  Collaborative mutagenicity testing, neurobehavioral toxicity, and hepato- and renal-toxicity studies on this mixture of 25 groundwater contaminants have been or are being investigated by the Health Effects Research Laboratories of the EPA. While most of the completed studies have yielded negative results for this mixture of groundwater contaminants, these toxic effects have been observed: (1) immuno-suppression in B6C3F1 mice as shown by stem cell proliferation, antibody formation (from challenge of sheep RBC), and host resistance from Plasmodium challenge; (2) inhibition of hepatic mitochondrial respiration; and (3) indication of enhancement of carbon tetrachloride hepatotoxicity in male Fischer-344 rats by the pretreatment of this mixture of groundwater contaminants. Planning is under way for future studies (toxicological interactions, chronic toxicity) on mixtures, and in particular for a simulated mixture of pesticides.

- Microencapsulation permits adding volatile or chemically-reactive compounds to the animal diet, rather than using the gavage route of exposure, and essentially eliminates problems with palatability. Feeding studies using chemicals that have been microencapsulated include trichloroethylene, methyl chloroform, and 2-ethylhexanol. Current studies include the demonstration of bioequivalence of microencapsulated citral; cis, trans and cis/trans dichloroethylene; and cinnamaldehyde. The feasibility of adopting microencapsulation for toxicology studies shows promise of being a more practical and natural method of exposing animals to volatile chemicals.

- Studies of the comparative toxicity and carcinogenicity of commonly occurring nickel compounds in the workplace have been designed to relate carcinogenic results to alterations in the immune system and tissue distribution of nickel, and to compare the relative toxicities of water-soluble and insoluble metal compounds. These studies will also permit a comparison of carcinogenicity results from the rodent model systems with epidemiologic results in humans. After 13 weeks of exposure in rats and mice, the primary target organ was lung after exposure to nickel oxide, nickel sulfate, or nickel subsulfide, with nickel sulfate being the more toxic compound. This correlates with increased water solubility. Two-year toxicology and carcinogenicity studies on each of these three are now in progress.

- An in vivo transplant model of Fischer rat leukemia has been developed and is being maintained by serial cell transfer, resulting in an assay capable of showing tumor expressions in only 2 months. The validity of this short-term model for predicting chemical leukemogens has been demonstrated retrospectively for three chemicals that were associated with leukemia in 2-year studies: pyridine, 2,4,6-trichlorophenol, and dichlorvos. Two chemicals exhibiting negative trends for leukemia in 2-year assays (2-ethoxyethanol and 4-hexylresorcinol) were also negative in the short-term assay. The leukemia transplant model has been responsive to these five chemicals, and appears reflective for the earlier detection of this tumor.

- Chemical disposition studies provide information on absorption, distribution, metabolism, and excretion that is then used in the design and/or interpretation of carcinogenicity results of select chemicals or chemical classes. These studies permit a good assessment of structure/activity relationships which influence chemical disposition or mechanisms of toxicity, and provide information to facilitate extrapolation of laboratory data to humans. In vitro methods are being used to study the metabolism of chemicals in human tissues to compare the metabolism to that in rat tissues. While the metabolic profile is generally similar, there are qualitative and quantitative differences between these two species. Other studies include the evaluation of the senescent changes in metabolism and their relationship to toxicity.

- Comparative toxicity studies of antiviral agents that appear to have potential for use in the treatment of AIDS are being conducted in rodents. Endpoints include general target organ toxicity as well as toxicity to the nervous and hematologic systems which have been shown to be damaged after antiviral treatment of AIDS patients. Drug blood concentrations are being measured to allow for comparison with concentrations seen in humans.

- Studies of the effects of chemicals on the immune system continue. Chemicals that affect immune responses, immunotoxicity, chemicals including therapeutic agents for treatment of AIDS, were investigated, and additional chemicals are being evaluated. Methods have been developed and experiments designed to evaluate the potential effects on the immune system which could result from occupational, inadvertent, or therapeutic exposure to drugs, environmental chemicals, or biological materials. Studies were done to develop and utilize B-cell maturation as an in vitro model to sequentially examine the cellular and molecular events associated with chemical-induced immunotoxicity. Other model systems were evaluated which would allow assessment of neutrophil and alveolar macrophage functions. Also investigated was the immunotoxicity associated with the tumorigenic process in mice that were exposed neonatally to diethylnitrosamine in combination with contaminated drinking water or with chemicals with potential therapeutic use in AIDS patients.

- As part of the effort to evaluate chemicals used or found in the semi-conductor industry, studies of the toxicity of inhaled arsine and gallium arsenide have been undertaken. A long-term inhalation exposure study of methylene chloride in female mice is under way to investigate the effects of chronic exposure and age on the pharmacokinetics of methylene chloride, on occurrence of oncogene activation, and on the relationship between cytotoxicity, cell proliferation, and tumorigenesis.

- Chemicals adversely affect development and reproduction. As a complement to our continuous breeding reproduction studies and to evaluate ovarian function as a target for toxic effects, the ovaries of female mice used in our continuous breeding studies are being evaluated by the NCTR. Results will be useful for developing and refining methods for examining the ovaries and for correlation of structural changes with functional changes observed in continuous breeding studies.

- Intramural projects continue in the areas of male fertility, postnatal toxicity, and teratology. In an effort to integrate animal model data with results in human semen evaluations conducted through NIOSH, the rabbit has been investigated as a model for comparison to the human, using ethylene dibromide. Studies have been conducted to characterize the effects of three chemicals on lactation and postnatal development. Studies were begun to investigate the potential of chemicals to adversely affect the developing immune system of mice, using TCDD and DES as prototype chemicals. The role of Sertoli cells in the expression of testicular toxicity continues to be investigated using cultured cells and chemicals which are either known to affect or not to affect testicular function.

- Investigations are under way on the role of cell proliferation in genesis of cancer, exploring the use of animal models in the initiation and promotion in chemical carcinogenesis, morphometric analysis of chemically-induced lesions, and use of magnetic resonance imaging to follow the progression or regression of chemically-induced lesions in live rodents. Differences in patterns of oncogene activation have been documented in naturally-occurring versus chemically-induced tumors in rodents. This work is being extended to determine the effect of different carcinogens on the activation of oncogenes, the role of oncogenes in the progression from hyperplastic to malignant lesions, and to characterize molecular lesions at the genomic level in chemically-induced tumors in different strains of rodents. The main purpose of these investigations is to further characterize the carcinogenic process and to help determine if the processes seen in rodent carcinogenesis are applicable to humans. Investigations are currently under way to determine the role of chemically induced cell proliferation in lung and liver tumors associated with carcinogens such as ethyl acrylate and methylene chloride.

- The use of selected in vitro genetic toxicity assays for predicting, retrospectively, rodent tumorigenicity is currently being extended to include in vivo cytogenetic effects. This evaluation will determine to what extent selected in vivo endpoints can complement the Salmonella mutagenesis assay.

Further efforts to evaluate and understand the relationships between genetic toxicity and carcinogenicity have clarified some of the discrepancies in the identification of genotoxic chemicals by showing the high (>90%) concordance between electrophilic substituents (structural alerts) of chemicals and the Salmonella mutagenesis assay. The value and importance of identifying mutagens is shown in the characteristics of the majority of human carcinogens: they possess structural alerts and are genotoxic in short-term assays.

o Another component of programmatic research relates to mutagenesis in mammalian germ cells. The effectiveness of x-rays at inducing mutations detectable in the electrophoretic specific locus assay has been clearly demonstrated and includes the induction of mutants at some loci for which mutants have not been previously observed. The human carcinogen chlorambucil was demonstrated to be a potent germ cell mutagen in the morphological specific locus assay. The frequency of mutants recovered is similar to or exceeds that seen for a very active mutagen, ethylnitrosourea. Adriamycin treatment gave negative results in the morphological specific locus assay and melphalan is being evaluated. The results of treating post-mating zygotes with mutagens have opened up several lines of study that include a unique stage of sensitivity, a new spectrum of developmental anomalies that appear to have a genetic basis, the recovery of morphological mutants that are mosaics, and a potentially useful method for inducing aneuploids.

o Progress has continued in the area of carcinogen metabolism. The metabolic capability of human cells in culture, used for screening for mutagenic chemicals, has been improved by the transfection of genes coding for two human cytochrome P-450 isozymes and epoxide hydrolase. The resultant modified human cells are 10 and 1000 times more sensitive to the mutagenic effects of benzo(a)pyrene and dimethylnitrosamine than are the parent cells.

o New rapid and efficient methods of cytology were developed and applied to meiosis (germ line differentiation) in yeast. These methods revealed that the pattern of chromosome behavior during yeast meiosis precisely parallels the same pattern in mammalian gametogenesis (spermatogenesis or oogenesis). A unique gene required for this germ line chromosome behavior was molecularly cloned from yeast and shown at the DNA sequence level to be evolutionarily conserved in higher organisms. This gene is required for chromosome synapsis, faithful segregation, and subsequent viability in the germ line but to be completely dispensable for somatic growth of yeast. Immunocytology of sera from humans with autoimmune or related diseases has revealed potentially similar patterns of recognition of nuclear structures in both yeast and mammalian cells. These sera are being further characterized to facilitate identification of conserved nuclear antigens which may serve as structural probes to study proper germ line chromosome behavior. Together, these observations indicate that the fundamental mechanisms of inheritance are remarkably similar at the cellular and molecular levels in yeast and in mammals. Thus, for the first time, there is good support for a non-animal (yeast) model of mammalian germ line chromosome behavior and inheritance.

o A system for prolonged incubation or culture of isolated renal tubules from a variety of mammalian species, including humans, for comparative studies is being developed and used. Isolated renal proximal tubules have been prepared from F344 rats and various specific measures of viability and functional capacity have been determined. These parameters are being developed for use as sensitive endpoints to study early events (and their progression) in chemically-induced nephrotoxicity at the molecular, subcellular, and cellular levels.

CARCINOGENESIS AND TOXICOLOGY EVALUATION BRANCH

CARCINOGENESIS AND TOXICOLOGIC EVALUATION BRANCH
Summary Statement

The Carcinogenesis and Toxicologic Evaluation Branch (CTEB) of the NIEHS conducts research designed to detect and characterize the toxic potentials of chemical agents. Although most of these studies are conducted in rodent species or other experimental model systems, the results are relevant to and are a major factor in estimating the toxic potentials of chemicals to humans. In addition to conducting chemical toxicity and carcinogenicity studies, the CTEB collaborates with other scientific staff at the Institute in developing and validating alternatives to standard toxicity tests and conducts biological and biochemical research in chemical toxicity.

The major effort of the CTEB staff during FY 1988 was the design, conduct, monitoring, evaluation, and reporting of toxicity and carcinogenicity studies completed off-site at contract laboratories approved to conduct NTP studies. These studies encompassed both prechronic (14-day), subchronic (90-day), and chronic (up to lifetime exposure) whole-animal tests employing morphological, clinical, and biochemical endpoints. Although many of these studies follow a standard protocol with regard to frequency of exposure and range of endpoints, each individual protocol is carefully designed to the chemical and physical properties of the test chemical, the needs of the requesting agency, and the usual route of exposure to the population at potential risk.

The Program Resources Group provides essential analytical chemistry and health and safety services for the toxicology and carcinogenesis studies conducted by the National Toxicology Program. Group staff procure, analyze, and monitor chemicals for purity, stability, and correct dose formulation. The Chemical Health and Safety office monitors all contract laboratories as well as each study within a facility for conditions which may adversely affect the proper research and study environment. Both programs are accomplished by branch staff and are supplemented by off-site resource contracts.

The Program Resource Group maintains a repository for over 1700 reference chemicals which are currently under study or which have completed studies in the various NTP programs. The Collaborative Services Group monitors Master Agreement Awards and is responsible for maintenance of the NTP Statement of Work and Master Agreements that enable laboratories to compete for Master Agreement Order contracts.

Extramural Research Highlights: Extramural research activities are conducted via contract mechanisms.

- The toxicity and carcinogenicity testing of approximately 250 chemicals is being performed under the NTP Master Agreement. This number includes chemicals in any of the stages from design through report preparation (CTEB staff).

- The Branch procured and/or analyzed 96 chemicals for the general *in vivo* chronic toxicology studies. In addition, 77 chemicals were obtained and analyzed for other programs within the National Toxicology Program such as teratology studies, immunotoxicology studies, reproductive toxicology studies, continuous breeding experiments, oncogene studies, AIDS-related drugs studies, and in-house DTRT studies. Services were provided for the analysis of bulk chemical and dosage formulations as well as evaluation of stability for both bulk chemicals and dosage formulations. Special studies were also conducted for chemical residue analysis in body tissues and fluids. Microencapsulation of study materials is actively being investigated as an alternative for administration of study chemicals in the feed rather than gavage of the neat chemical. Support was also provided to the Cellular and Genetic Toxicology Branch by analyzing over 60 chemicals for purity and identity. The Chemistry Group provided staff support for data auditing activities of the DTRT. This included review of completed studies to assure the chemistry performed in support of these studies was adequate and accurately reported.

- Recent NTP carcinogenesis and toxicologic studies have used, where possible, the route of exposure most commonly experienced by the human population. Since skin comes into contact with many chemicals, this route of exposure has been used to study chemical toxicity with increasing frequency. There are currently over 20 chemicals being evaluated using the standard NTP toxicology and carcinogenesis design. In addition, to obtain a more complete toxicologic profile for compounds evaluated in a skin exposure protocol, special studies are conducted to determine percutaneous absorption and chemical disposition. These data are used to set dose exposure levels, to determine target tissues, and to help provide an explanation of chemical effect. In addition to the standard evaluations, studies designed to compare mouse strain sensitivity using the mouse-skin initiation/promotion protocol have been completed and the data are in review at NTP. On a selective basis, this design has and continues to be used to determine initiation and promotion potential. (Dr. Eastin)

- Development and use of a system for the prolonged incubation or culture of isolated renal tubules from a wide variety of mammalian species, including humans, for comparative studies is being conducted under contract. Isolated renal proximal tubules have been prepared from F344 rats and various specific measures of viability and functional capacity have been determined. These parameters are being developed for use as sensitive endpoints to study early events (and their progression) in chemically-induced nephrotoxicity at the molecular, subcellular, and cellular levels. (Dr. Rauckman)

- Processing and manufacturing of nickel has been associated with an increased risk of respiratory tract cancer in humans, but the causative agent and risk to individual nickel compounds has not been identified. The Program is studying the comparative toxicity and carcinogenicity of commonly occurring nickel compounds in the work place including nickel oxide, nickel sulfate, and nickel subsulfide. These studies are designed to relate carcinogenic results to alterations in the immune system and tissue distribution of nickel, and to compare the relative toxicities of water soluble and insoluble metal compounds. These studies are also designed as a model for comparing carcinogenicity results from NTP animal model systems (F344/N rats and B6C3F1 mice) with results in humans. After 13 weeks of initiation exposure in F344/N rats and B6C3F1 mice, the primary target organ was lung after exposure to nickel oxide, nickel sulfate, or nickel subsulfide. Nickel sulfate was more toxic than the other two nickel compounds, which correlates with increased water solubility. Two-year toxicity and carcinogenicity studies are now in progress. (Dr. Dunnick).

- Comparative toxicity studies of promising antiviral agents used in the treatment of AIDS are being conducted in rodents. Endpoints being evaluated include general target organ toxicity as well as special testing of toxicity to the nervous system and to the hematologic system (systems which have been shown to be damaged after antiviral treatment of AIDS patients). Drug blood concentrations are being measured to allow for comparison with concentrations seen in man. AIDS infection results in destruction of T-cells. In order to evaluate more completely the toxicity of antiviral agents, studies of toxicity will also be conducted in immunocompromised animals such as a retrovirus-infected mouse or a nude mouse. The NIEHS has set up an AIDS advisory panel composed of representatives from academia, industry, and government which reviews the test protocols and helps select the most promising therapies for evaluation.

  The NIEHS is collaborating with NIAID and NCI on a workshop to discuss and develop methods for toxicity testing of cytokines (e.g. interferon) used in the treatment of AIDS. This workshop is planned for August 1988. (Dr. Dunnick)

- Developmental chemistry work on formulating a 25-chemical mixture in deionized water was conducted between July 1986 and June 1987. The scope of this initial phase of the studies included the formulation of a chemical mixture containing 25 frequently detected groundwater contaminants, analyses of the purity of each of the 25 chemicals, and the studies of stability of the mixture under normal laboratory and storage conditions. A technically achievable mixture of 25 chemicals in deionized water with reasonable stability was prepared. The animal toxicity studies (a pilot palatability and mortality study and a 3-month subchronic toxicity study with a 3-month recovery period in rats and mice) on the mixture were awarded in June 1987. Measured or observed parameters include clinical signs, mortality, water and food consumption, body and organ weights, clinical and anatomic pathology, neurobehavioral tests, sperm morphology and vaginal cytology evaluation (SMVCE), and cytogenetics. (Dr. Yang)

- The role of the quantity and quality of diets and the susceptibility of animals to cancer has long been recognized. Many investigators showed that under-feeding of animals causes a reduction in spontaneous tumor incidence. For this reason we plan to investigate the influence of feed restriction on the outcome of toxicity and carcinogenicity studies with four chemicals in rats and mice. The four chemicals are t-butylhydroquinone, scopolamine hydrobromide, butyl benzyl phthalate, and salicylazosulfapyridine. In these studies two feeding regiments (ad libitum or restricted) will be used. Restricted animals will be offered a daily allotment of food equivalent to 75% to 80% of the amount consumed by the ad libitum animals. (Dr. K.M. Abdo)

Intramural Research Highlights: Intramural research is conducted at the NIEHS by CTEB staff and collaborating scientists.

- Microencapsulation is a process for completely enveloping tiny masses of solid particles or liquid droplets in a protective coating which separates the substance from its environment. The use of microencapsulated chemicals for toxicology studies presents a number of advantages, i.e., it permits testing volatile or chemically-reactive compounds in the animal diet, minimizes problems with palatability, etc. Volatile and/or reactive chemicals have been encapsulated using a starch, gelatin, or gelatin/sorbitol matrix and determined to be stable when mixed with rodent feed. Bioequivalence studies in rats of microencapsulated trichloroethylene, methylchloroform, and 2-ethylhexanol indicates no significant difference in availability from neat test chemicals after oral administration. Feeding studies using these microencapsulated chemicals have been successfully completed. Current studies include the demonstration of bioequivalence of microencapsulated citral, cis, trans and cis/trans dichloroethylene, and cinnamaldehyde. (Dr. Jameson, Dr. Goehl, and Dr. Melnick)

- The Program is exploring the feasibility of adopting microencapsulation for toxicology studies as a more practical and natural method of exposing laboratory animals to volatile, reactive, and/or unpalatable chemicals. In dosed feed studies of microencapsulated 2-ethylhexanol and of microencapsulated methyl chloroform (1,1,1-trichloroethane) in Fischer 344 rats, these compounds were stable in feed and were consumed at doses which are sufficient for toxicologic evaluations. Since microencapsulation did not interfere with the bioavailability of these compounds in rats, this technique appears to be a suitable alternative for studying the oral toxicologic properties of volatile chemicals in laboratory animals. (Dr. Melnick)

- Triethanolamine is widely used in cosmetic products such as creams, skin cleaners, and shampoos. In a 14-day dermal study, triethanolamine was found to be more toxic to the skin of rats than mice. In a dermal absorption study, triethanolamine was found to be more rapidly absorbed from the skin of B6C3F1 mice than F344 rats. The difference in absorption of triethanolamine in rats and mice may be an important factor in the species difference in the dermal toxicity of this chemical. (Dr. Melnick)

- Inhibition of mitochondrial respiration by a mixture of 25 groundwater contaminants is being investigated. The chemical mixture which includes heavy metals, aromatic hydrocarbons, and halogenated solvents was administered in drinking water to F344 rats for 14 days (see Yang, below). The most apparent biochemical change in the liver of treated animals was an inhibition of mitochondrial respiration. The total chemical mixture inhibited state-4 (oxidation) and state-3 (phosphorylation) respiration rates of isolated rat liver mitochondria at 1/500 the concentration used in the drinking water study. Neither a mixture of the organic components nor the individual heavy metals inhibited the mitochondrial state-3 or state-4 respiration rates at the effective concentration of the total mixture. The inhibition of mitochondrial respiration by the chemical mixture is probably due to a synergistic effect of some of its components. (Dr. Melnick)

- An in vivo transplant model of Fischer 344 rat leukemia was developed and has been maintained by serial cell transfer into syngeneic recipients every 60-90 days. The time to expression of the tumor was reduced from 18 months to 2 months. Tumor marker enzymes in enriched mononuclear cells obtained from peripheral blood samples were diagnostic for leukemia prior to the expression of other clinical indices. The validity of the short-term model for predicting the chronic potency of chemical leukemogens was evaluated with chemicals previously tested in NCI/NTP 2-year toxicity and carcinogenicity studies. Chemicals that were associated with positive trends for leukemia in 2-year tests (pyridine, 2,4,6-trichlorophenol, and dichlorvos) were also positive in the short-term assay, and those associated with negative trends for leukemia in 2-year tests (2-ethoxyethanol and 4-hexylresorcinol) were also negative in the short-term assay, demonstrating that the leukemia transplant model is accurate for a broad range of chemicals, and is efficient for the early detection of this tumor. A structure-activity relationship between the dimethyl esters of phosphoric acid found in dichlorvos and five other structurally-similar chemicals from the NTP chronic data base, and the occurrence of mononuclear cell leukemia was observed. The proto-oncogene c-FMS was expressed by mononuclear cells isolated from the spleen and blood of rats with spontaneous leukemia, and in the same cells from rats transplanted with leukemia. (Dr. Dieter and Dr. French)

- Toxicological studies of metallic salts are being conducted to support and extend results from contract studies. Blood and target organ concentrations of metal residues are used to measure disposition and steady state concentrations. Twenty day i.p. injection studies with sodium chromate, zinc potassium chromate, and chromium carbonyl were conducted on female mice to compare the genotoxic effects of the hexavalent salts with their potential myelotoxic effects, and to determine which salt(s) require further evaluation in 2-year tests. Studies of the absorption and toxicity of ferric ammonium ferrocyanide will be initiated to determine the feasibility of further toxicological studies of this insoluble blue pigment. (Dr. Dieter)

- A series of in vivo and in vitro investigations are in progress to address questions regarding cellular and molecular processes responsible for the induction of lung and liver tumors in mice by a model chlorinated hydrocarbon, methylene chloride. Previous NIEHS studies have shown that methylene chloride causes dose dependent increases in cancers of the lung and liver of female mice (NTP Technical Report #306) at doses which are otherwise relatively non-toxic to the animals. Consequently methylene chloride-induced carcinogenesis in mice serves as an ideal experimental model to simultaneously test specific hypotheses regarding chemically-induced cancer in these two organs.

  We have begun a two year chronic study in which the primary objective is to identify, characterize, and compare the oncogenes expressed in tumors isolated from control versus methylene chloride-treated mice. This research is aimed at resolving whether the chlorinated hydrocarbon introduces new mutations into DNA, increases the expression of endogenous molecular lesions, or both, or neither. Additional experiments are being conducted to probe the putative involvement of mitogenic-, genotoxic-, and immunotoxic activities in methylene chloride-induced carcinogenicity in order to provide complimentary information for interpreting the molecular biology results. (Dr. Frank Kari)

- The National Toxicology Program has reported on the comparative evaluation of the carcinogenicity of two nitrophenylenediamines (HC Blue 1 and HC Blue 2). These studies revealed incongruities in their carcinogenic activities which would not be expected based on their structural similarity. We are currently following-up these observations with a combination of in vitro and in vivo experiments to elucidate biochemical mechanisms responsible for the different outcomes in the bioassays.

Preliminary studies of the comparative metabolism of HC Blue 1 and HC Blue 2 have been completed in bacteria, isolated hepatocytes, and in vivo. Our results indicate that isolated hepatocytes serve as an appropriate model for metabolism because the metabolic profiles of both compounds obtained in vivo are identical to those seen in isolated hepatocytes. Our results also reveal significant quantitative and qualitative differences in the metabolic profile of the carcinogen HC Blue 1 compared to that of the non-carcinogen HC Blue 2. These differences in metabolism may be responsible for the discordance in the observed carcinogenicity and genotoxicity of these compounds. We are currently isolating and identifying metabolites from both compounds to identify unique metabolites formed from HC Blue 1 but not from HC Blue 2. Additionally, we have undertaken in vivo and in vitro studies to assess DNA adduct formation from these two chemicals. (Dr. Frank Kari)

- Inter-branch collaborative studies on the the 25-chemical mixture mentioned previously were initiated within NIEHS. The entire project consists of: (1) palatability and range-finding studies in Fischer 344 rats and B6C3F1 mice; (2) short-term (14-day) immunotoxicity screen in female B6C3F1 mice, and developmental toxicity screen in pregnant Fischer 344 rats; (3) a 13-week immunotoxicity study in female B6C3F1 mice, and a 13-week male reproductive toxicity study in B6C3F1 mice; (4) biochemical studies on hepatic mitochondrial respiration and enzymatic activities in male Fischer 344 rats; (5) myelotoxicity studies in B6C3F1 mice; (6) continuous breeding reproductive toxicity studies in CD mice and CD rats; (7) in vivo cytogenetic studies in Fischer 344 rats and B6C3F1 mice. Mutagenicity testing, neurobehavioral toxicity, and hepato- and renal toxicity studies on this mixture of 25 groundwater contaminants have been or are being investigated by colleagues from the Health Effects Research Laboratories of the EPA. (Dr. Yang)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES 21050-05 CTEB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Evaluation of Microencapsulation As Means to Administer Chemicals in Feed.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: C. W. Jameson Head, Program Resources Group CTEB NIEHS
Others: T. J. Goehl Chemist CTEB NIEHS
R. L. Melnick Head, Experimental Toxicology Unit CTEB NIEHS
B. J. Collins Chemist CTEB NIEHS
T. Gorski Visiting Fellow CTEB NIEHS
A. Greenwell Technician CTEB NIEHS
F. Harrington Technician CTEB NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Carcinogenesis and Toxicology Evaluation Branch

SECTION

Program Resources Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.25	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Microencapsulation is a process for completely enveloping tiny masses of solid particles, or liquid droplets in a protective coating which separates the substance from its environment. The use of microencapsulated chemicals for toxicology studies presents a number of advantages, i.e. it permits testing volatile or chemically reactive compounds in the animal diet, minimizes problems with palatability, etc. Volatile and/or reactive chemicals have been encapsulated using a starch, gelatin or gelatin/sorbitol matrix and determined to be stable when mixed with rodent feed. Relative bioequivalence in rats of the microencapsulated trichloroethylene, 1,1,1-trichloroethane and 2-ethylhexanol compared to the neat test material indicates no significant difference in absorption after oral administration. Palatability studies using the microencapsulated trichloroethylene, 1,1,1-trichloroethane and 2-ethylhexanol have been successfully completed. Current studies include the demonstration of bioequivalence of microencapsulated citral, cis-dichloroethylene, trans-dichloroethylene and cis/trans-dichloroethylene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-21076-05 CTEB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cellular Biochemistry Studies on Chemicals Selected for Evaluation by NTP

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael P. Dieter	Physiologist	DTRT/CTEB	NIEHS
Others:	C.W. Jameson	Chemist	DTRT/CTEB	NIEHS
	M.D. Shelby	Head, Mammalian Mutagenesis	DTRT/CGTB	NIEHS
	G.A. Boorman	Pathologist	DTRT/CPB	NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

CTEB

SECTION

N.A.

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The effect of inorganic or organic metals and metal complexes is of particular interest because of their prevalence in drinking water and industrial processes, use as constituents in anticancer and antihelminthic drugs, and their diverse target organ toxicities. Toxicological studies of various, selected metallic salts are being conducted to support and extend the results obtained at contract test facilities. Blood and target organ levels are measured to determine the disposition and steady state concentrations of the metal residues. Cellular biochemical responses provided sensitive indices of target organ toxicity that often preceeded clinical signs or microscopic evidence of pathology. Metal salts that have been studied include mercuric chloride, nickel sulfate, and titanocene dichloride. Further studies are underway with sodium chromate, zinc potassium chromate, and chromium carbonyl in female mice to compare the genotoxic and myelotoxic effects of the different hexavalent salts. The effects of 20-day i.p. injections on micronuclei, on bone marrow stem cell proliferation rates, and on cellular biochemistry are being conducted. Studies of the absorption and toxicity of the organoferric salts, ferrocene and ferric ammonium ferrocyanide, are being initiated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21078-05 CTEB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Bioavailability and Toxicity Studies of Microencapsulated Chemicals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ronald L. Melnick	Toxicologist	DTRT/CTEB	NIEHS
	Thomas Goehl	Chemist	DTRT/CTEB	NIEHS
	C.W. Jameson	Chemist	DTRT/CTEB	NIEHS
Others:	Brad Collins	Chemist	DTRT/CTEB	NIEHS
	Frank Harrington	Bio. Lab. Tech	DTRT/CTEB	NIEHS
	Arnold Greenwell	Biologist	DTRT/CTEB	NIEHS
	Tad Gorski	Visiting Fellow	DTRT/CTEB	NIEHS

COOPERATING UNITS *(if any)*

Midwest Research Institute, Kansas City, MO

LAB/BRANCH

Carcinogenesis and Toxicologic Evaluation Branch

SECTION

Experimental Toxicology Unit: Program Resources Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The National Toxicology Program is exploring the feasibility of adopting microencapsulation as an alternative means of incorporating volatile, reactive, and/or unpalatable chemicals into animal feed for toxicologic studies. Oral (dosed-feed) toxicology studies are performed on those chemicals which are demonstrated to be adequately stabilized in the microcapsules, but released into the gastrointestinal tract to allow biological availability. Trichlroethylene, 2-ethyhexanol, and methyl chloroform have been stabilized in microcapsules and evaluated for palatability and bioavailability in Fischer 344 rats. In each case, the absorption of the microencapsulated chemical was equivalent to that of the neat compound administered by gavage to Fischer 344 rats. In 14-day repeated-dose studies in F344 rats, similar toxic effects were produced by microencapsulated chemical given in feed as that of the neat chemical dissolved in corn oil administered by gavage. These studies indicate that microcapsulation can provide an excellent alternative method for studying the oral toxicological properties of volatile chemicals in laboratory animals. Future work will involve bioequivalence studies and toxicologic characterization of other microencapsulated chemicals.

PHS 6040 (Rev 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21079-05 CTEB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of Di(2-ethylhexyl)phthalate Hepatotoxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ronald L. Melnick	Chemist	DTRT/CTEB	NIEHS
Others:	Konrad Tomaszewski	Visiting Fellow	DTRT/CTEB	NIEHS
	Walter Jenkins	Biologist	DTRT/CTEB	NIEHS
	Brenda Ferguson	SIS	DTRT/CTEB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Carcinogenesis and Toxicologic Evaluation Branch

SECTION

Experimental Toxicology Unit

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In a two-year study conducted by the National Toxicology Program, the industrial plasticizer, di(2-ethylhexyl)phthalate (DEHP), was found to be hepatocarcinogenic in B6C3F$_1$ mice and F344 rats. Since DEHP induces peroxisome proliferation but is not itself a mutagen, it has been suggested that carcinogenicity of this chemical may be due to excessive peroxisomal production of H_2O_2. The effects of DEHP on hepatic peroxisomes in rats and mice have been investigated. Peroxisomal acyl CoA oxidase, the first enzyme in the β-oxidation sequence, was established as the most suitable marker for hepatic peroxisome proliferation. Maximal peroxisomal induction by DEHP occurred at a dose of 2 g/kg/day, and the no-observable effect dose was 0.6 g/kg/day. Kinetic data on the rates of formation of H_2O_2 during peroxisomal oxidation of palmitoyl CoA, and of degradation of H_2O_2 by catalase were used to estimate in vitro steady state H_2O_2 concentrations during peroxisomal β-oxidation. Increases in steady state [H_2O_2] in liver homogenates of rats treated with DEHP and other peroxisome proliferators (nafenopin and di(2-ethylhexyl) phthalate) correlated well with the carcinogenic potential of these chemicals. These findings are consistent with an involvement of peroxisome proliferation in hepatocarcinogenesis. Peroxisomal enzymes activities were also found to increase in primary hepatocyte cultures incubated with mono(2-ethylhexyl) phthalate (the primary metabolite of DEHP), nafenopin, and clofibric acid. Furthermore, there was an increase in conjugated dienes, an indicator of lipid peroxidation, in treated hepatocytes. Thus, oxidative stress was associated with peroxisome proliferation in rodent hepatocytes. Studies have been initiated to characterize hydrogen peroxide mediated lipid peroxidation in isolated hepatocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21095-02 CTEB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of In Vitro Propagated F344/N Mononuclear Cell Lines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	John E. French	Physiologist	DTRT/CTEB	NIEHS
Others:	M.P. Dieter	Physiologist	DTRT/CTEB	NIEHS
	S. Gangjee	Visiting Scientist	DTRT/CTEB	NIEHS
	S.A. Stefansky	Pathologist	DTRT/CPB	NIEHS

COOPERATING UNITS (if any)

Chemical Pathology Branch, DTRT, NIEHS

LAB/BRANCH

Carcinogenesis and Toxicologic Evaluation Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The high background incidence of mononuclear cell leukemia (MNCL) in Fischer 344 rats (20 to 30%) confounds the evaluation and interpretation of possible chemical treatment related incidence of MNCL in two-year chronic toxicology and carcinogenesis studies. A F344 rat leukemia transplant model has been developed to characterize the biology of this rodent leukemia and to study the tumor biology and determine how chemical treatment effects disease expression. The development and use of in vitro propagated F344/N mononuclear leukemic cells will enhance: (1) development of monoclonal antibodies unique to MNCL for diagnostic purposes and staging of the disease, (2) the use of currently available rat cell surface antigen and receptor data to known cytochemical, morphological and cell biochemistry data and the determination of leukemic cell origin and functional lineage, and (3) the use of in vitro tests to determine the toxicity and carcinogenicity of chemicals under study in the in vivo MNCL transplant model.

To date several hybriloma cell lines have been developed that have been determined to secrete antibodies that may be unique to these leukemic cells. Cell separations have been developed that allow separation of different leukemia cell types. These different subsets of leukemia cells have been propagated in vivo through several passages and have been cryopreserved for future characterization, (including monoclonal antibody analysis), and in vitro culture as the methods for diffusion chamber, soft agar, and Dexter type cultures and culture conditions are refined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21096-02 CTEB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Identification and Isolation of c-fms Protooncogene From F344/N Rat Leukemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John E. French	Physiologist	DTRT/CTEB	NIEHS
Others:	C. Walker	Chemist	DIR/LPP	NIEHS
	S. Gangjee	Visiting Scientist	DTRT/CTEB	NIEHS
	S.A. Stefansky	Pathologist	DTRT/CPB	NIEHS

COOPERATING UNITS *(if any)*

Laboratory of Pulmonary Pathobiology, DIR, NIEHS
Chemical Pathology Branch, DTRT, NIEHS

LAB/BRANCH

Carcinogenesis and Toxicologic Evaluation Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The proto-oncogene, c-fms, has been described in both mature cat macrophages and undifferentiated mouse and human tumors, and normal human bone marrow cells. The c-fms proto-oncogene is expressed during human monocyte differentiation and the c-fms product has been related to the receptor for mononuclear phagocyte growth factor (CSF-1).

The purpose of this investigation is: (1) to screen for the presence of the c-fms proto-oncogene in the in vivo serially transplanted F344/N mononuclear leukemic cell lines and, (2) to initiate studies on the isolation and expression of the gene product. This will provide insight on the origin of the tumor and aid in providing diagnostic criteria for the tumor. In addition, this tumor line may provide a valuable source of genetic material for investigations into the molecular biology of the c-fms protooncogene and its potential effect on MNCL expression. Our investigation indicates the presence of c-fms oncogene isolated from both spontaneously occurring MNCL tumors and these leukemic cells appear to have a normal karyotype (except for an unusual subterminal x-chromosome variant) and possess hematopoietic differentiation antigens of both monocytes and lymphocytes, in vivo transplanted MNCL tumor lines.

In situ hybridization technique using monolayer of heterogeneous leukemic cell population are being pursued to allow identification of c-fms in specific cell types.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21097-02 CTEB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Evaluation of Chemical Myelotoxicity Using an In Vivo Leukemia Transplant Model

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael P. Dieter	Physiologist	DTRT/CTEB	NIEHS
Others:	J.E. French	Physiologist	DTRT/CTEB	NIEHS
	C.W. Jameson	Chemist	DTRT/CTEB	NIEHS
	G.A. Boorman	Pathologist	DTRT/CPB	NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

CTEB

SECTION

N.A.

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Up to 30% of the Fischer 344/N strain of rats used in the NTP 2-year toxicity and carcinogenicity studies develop spontaneous leukemia commencing at about 18 months, which reduces the sensitivity for the detection of chemical leukemogenesis. An in vivo cell transplant model was developed to transfer leukemic spleen cells from a rat with spontaneous leukemia into young, healthy recipient rats. After manifestation of the disease, leukemic spleen cells from those rats were again used for tranplantation to maintain the leukemic cell line in vivo. The morphological and biochemical responses in organs and cells from the transplanted rats were characterized to better discriminate between age-induced and chemically-enhanced leukemia. The tumor morphology, cellular biochemistry, and oncogenetic expression in the spontaneous and transplanted cases were similar, but the time to tumor was reduced from 18 months to 2 months. The accuracy and sensitivity of the transplant model for predicting the long-term leukemogenic potency of chemicals was confirmed in short-term assays with two chemicals (2-ethoxyethanol, 4-hexylresorcinol) that had decreased, and three chemicals that had increased (pyridine, dichlorvos, 2,4,6-trichlorophenol) the prevalence of leukemia in previous 2-year toxicity and carcinogenicity studies. Additional studies with this transplant model revealed structure-activity relationships between chemicals containing dimethyl esters of phosponic acid and the enhancement of leukemia, or between chemicals containing the glycol ether structure and the repression of leukemia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21102-02 CTEB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Dermal Absorption of Diethanolamine and Triethanolamine in Rats and Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Ronald L. Melnick	Chemist	DTRT/CTEB	NIEHS
Others:	Arnold Greenwell	Biologist	DTRT/CTEB	NIEHS
	Frank Harrington	Biologist Lab. Tech	DTRT/CTEB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Carcinogenesis and Toxicologic Evaluation Branch

SECTION

Experimental Toxicology Unit

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.1	.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Diethanolamine (DEA) and triethanolamine (TEA) are widely used in cosmetic products such as creams, skin cleaners, and shampoos. In a 14-day repeated-dose study conducted by the National Toxicology Program, TEA was found to be more toxic to the skin of rats than mice after dermal application. Dermal absorption studies of TEA in F344 rats and $B6C3F_1$ mice were initiated to help explain species differences in sensitivity to this chemical. The interscapular area of male rats and mice were clipped, and screened rings were mounted over the intended site of chemical application. ^{14}C-TEA dissolved in acetone was applied within the tissue caps to rats and mice. Blood samples were taken at eight time points over a 48 hour period after dosing, oxidized to CO_2, and assayed for ^{14}C by liquid scintillation counting. Radioactivity in urine, feces, tissue caps and skin sections from the site of application were also counted. TEA was absorbed after dermal application in rats and mice; however, the rate of absorption was greater in mice and the level of chemical retained at the site of application was greater in rats. Similar comparative dermal absorption studies of DEA in rats and mice are planned.

PHS 6040 (Rev 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21108-01 CTEB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical Toxicology of a Mixture of 25 Groundwater Contaminants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Raymond Yang	Chemist	DTRT/CTEB	NIEHS
	Ronald Melnick	Toxicologist	DTRT/CTEB	NIEHS
	C.W. Jameson	Chemist	DTRT/CTEB	NIEHS
Others:	Hamid Kermani	Visiting Fellow	DTRT/CTEB	NIEHS
	Frank Harrington	Biologist Lab. Tech	DTRT/CTEB	NIEHS
	Arnold Greenwell	Biologist	DTRT/CTEB	NIEHS
	Walter Jenkins	Biologist	DTRT/CTEB	NIEHS
	Brenda Ferguson	SIS	DTRT/CTEB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Carcinogenesis and Toxicologic Evaluation Branch

SECTION

Experimental Toxicology Unit

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

  ☐ (a1) Minors

  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A mixture of 25 groundwater contaminants identified in and around hazardous waste sites was formulated in water at various multiples of the average concentrations found in analytical surveys of ground water for water soluble compounds or at various dilutions of the 90% saturation level of the poorly soluble compounds. This mixture which contained heavy metals, aromatic hydrocarbons, and halogenated solvents was administered in drinking water or by gavage to Fischer 344 rats for 14 days. Evaluations were made of the effect of this mixture on hepatic biochemical parameters including mitochondrial respiration, lipid peroxidation, and levels of cytochrome P-450, aryl hydrocarbon hydroxylase, and glutathione-S transferase. A decrease in mitochondrial state-3 respiration (phosphorylation of ADP) was the most notable effect of administration of this chemical mixture. In vitro studies are in progress to identify the specific effects of the components of this mixture on mitochondrial respiration. Preliminary data indicate that cadmium was the most potent inhibitor of ADP phosphorylation in the mixture; however, inhibition occured at lower concentrations by the mixture than that caused by cadmium. An additive or possibly a synergistic effect of components of this mixture on mitochondrial oxidative phosphorylation is suggested.

CELLULAR AND GENETIC TOXICOLOGY BRANCH

CELLULAR AND GENETIC TOXICOLOGY BRANCH
Summary Statement

The Cellular and Genetic Toxicology Branch in the Division of Toxicology Research and Testing conducts studies that are generally related to the development, evaluation and use of in vitro and in vivo short-term tests to assess the genetic toxicity of chemicals. The Branch also conducts research on fundamental aspects of genetics and mutagenesis. These activities are diverse in scope but address important questions related to the understanding of genetic processes and the mechanisms and consequences of genetic injury.

The evaluation of the use of selected in vitro genetic toxicity assays for predicting rodent tumorigenicity reported in Science (236: 233, 1987) is currently being extended to include in vivo cytogenetic effects. The purpose of this evaluation, utilizing the same 73 chemicals reported above and tested under code, is to determine to what extent selected in vivo endpoints can complement the Salmonella mutagenesis assay. This complementation includes both their possible identification of actual mutagens and of mutagenic noncarcinogens. The in vivo cytogenetic assays will be evaluated for the ability to complement the Salmonella mutagenesis assay in a two test combination. While these studies are in progress, there have been further efforts to evaluate and understand the relationships between genetic toxicity and carcinogenicity. These studies (reported in Mutation Research 204: 1-115, 1988, and commented on in Nature 332: 782-783, 1988), have clarified some of the discrepancies in the identification of genotoxic chemicals by showing the high (>90%) concordance between electrophilic substituents (structural alerts) of chemicals and the Salmonella mutagenesis assay. The value and importance of identifying mutagens is shown in the characteristics of the majority of human carcinogens identified as Class I by the International Agency for Research on Cancer, which possess structural alerts and are genotoxic in short-term assays.

The major activity in the mouse bone marrow cytogenetics and the Drosophila mutagenesis projects has been the continued testing of the 73 chemicals used by this Branch in the assessment of in vitro short-term tests. All these chemicals have now either been tested or are on test in these assays. Meanwhile, the rodent bone marrow micronucleus assay has received additional attention with two new contracts awarded. In vivo cytogenetic studies were completed on several compounds of special interest to the NTP. These included butadiene, chloroprene and isoprene, the human carcinogens 4-aminobiphenyl, melphalan, and treosulphan, and the formulated complex mixture under study by the NTP. In addition, micronucleus results from blood smears taken during the prechronic and chronic toxicity studies are now beginning to appear in the NTP Technical Reports.

As a result of the Branch's evaluations, genetic toxicology has been accorded a more prominent role in the NTP Technical Reports. This has included moving the data tables from the appendix to the results section, adding detailed methods sections for the genetic toxicity assays, and greater emphasis on genetic toxicity information in the body of the Technical Reports.

An important component of the programmatic research of the Branch relates to mutagenesis in mammalian germ cells. The effectiveness of x-rays at inducing mutations detectable in the electrophoretic specific locus assay has been clearly demonstrated and includes the induction of mutants at some loci for which mutants have not been previously observed. The human carcinogen chlorambucil was demonstrated to be a potent germ cell mutagen in the morphological specific locus assay. The frequency of mutants recovered is similar to or perhaps exceeds that seen for the supermutagen, ethylnitrosourea. Adriamycin treatment gave negative results in the morphological specific locus assay and melphalan currently is on test. The results of treating zygotes (maternal exposure 1-hour post-mating) with mutagens have opened up several interesting lines of study that include a unique stage of sensitivity, a new spectrum of developmental anomalies that appear to have a genetic basis, the recovery of morphological mutants that are mosaics, and a potentially useful method for inducing aneuploids and recovering them for analysis.

Studies relating to the induction of aneuploidy and mechanisms of chromosome segregation have continued to be addressed using the yeast Saccharomyces cerevisiae. A highly sensitive system based on chromosome loss in mitotically growing cells has been developed and validated in two laboratories. Results with 16 chemicals tested under code by the laboratories showed excellent agreement. Another system for detecting chromosome gain in mitotic and meiotic cells has also been developed and is being validated. A unified system for detecting chromosome gain and loss is under development. Among the many chemicals that are strong inducers of aneuploidy are aprotic polar solvents such as acetone, acetonitrile, and 2,5-hexanedione; several chemicals are only effective as mixtures. Some of the solvents are also known to be neurotoxins. Based on measured effects on tubulin aggregation in vitro, the solvents appear to act at the level of microtubules. The in vivo effects on the induction of aneuploidy are currently being examined in Drosophila.

Progress has continued in two areas of carcinogen metabolism which are relevant to the long-term goals of CGTB. First, the metabolic capability of human cells in culture, which are used for genetic toxicity screening, has been improved by the transfection of gene coding for two human cytochrome P-450 isozymes and epoxide hydrolase. The human cells containing the transfected genes are 10 and 1000 times more sensitive to the mutagenic effects of benzo(a)pyrene and dimethylnitrosamine than are the parent cells. Cytofluorographic methods of detecting and isolating cells with enhanced metabolic capability have also been developed. The acquisition of replicating cells with metabolic capability will allow long-term, low-dose experiments to be conducted so that genotoxic effects can be measured with minimal cytotoxicity. The second accomplishment was that of comparing human liver and kidney cell metabolism to the respective rodent cell metabolism of the model carcinogens 2-acetylaminofluorene and benzo(a)pyrene. Interindividual variation in human hepatocyte and kidney cell metabolism of both chemicals was observed and the variation was greater with hepatocytes than kidney cells. For 2-acetylaminoflourene all human cells, except from one specimen, were more active metabolically than the respective rat cells, whereas rat cells showed about the same level of metabolism of benzo(a)pyrene as the average human cells. The results suggest that the range of human interindividual variation and human-rodent species differences can vary with the chemical being studied.

The mechanisms of glutathione and other thiol mutagenicity in Salmonella have been investigated, glutathione is metabolized to a dipeptide by gamma-glutamyltranspeptidase (GGT), an enzyme found in high levels in the kidney. The resulting cysteinylglycine spontaneously oxidizes to form hydrogen peroxide which is mutagenic and toxic via its breakdown to oxygen radicals. These reactions may be relevant to the progression of preneoplastic liver foci to tumors because these foci tend to have high levels of GGT. Therefore, there would be a high level of mutagenic oxygen radicals generated in these foci by the reaction of GGT with normal cellular levels of glutathione. Other thiols, such as cysteine, also appear to produce hydrogen peroxide when they autoxidize. The mutagenicity of sodium bisulfite, a ubiquitous food additive, is also mediated by free radicals produced by bisulfite as it autoxidizes.

Progress has been made in three areas of our study on the regulation of retrotransposition/retrovirus integration. First, the development of a retrovirus packaging/vector system in which integration is restricted in mouse cells expressing the Fv-1^b allele but not the Fv-1^n allele have been completed. Vector/packaging systems with different Fv-1 allele sensitivities are under construction. Secondly, the analysis of the Fv-1 tropism determinants of endogenous proviruses has continued. The Fv-1^n determinant is carried by the ecotropic provirus whereas the Fv-1^b tropism determinant is a property of a moderately abundant family (approximately 30 members) of endogenous MuLV-related proviral genomes. Most xenotropic proviruses appear to have neither specificity. Thirdly, a collaborative study of the regulation of the Fv-1 gene was begun. Cell cultures derived from a common progenitor have been identified in which the Fv-1 gene is phenotypically silent in some clones and expressed in other clones. Preliminary evidence suggests that this will provide a model system for further analysis of the Fv-1 gene, including molecular cloning.

The common bakers yeast has been developed as a non-animal model for studying genotoxic damage to either somatic or to germ line genetic inheritance during mammalian growth and reproduction. A rapid, inexpensive, and reliable test system for mutation and retroviral activity has been developed and calibrated using this non-animal system.

First, new rapid and efficient methods of cytology were developed and applied to meiosis (germ line differentiation) in yeast. These methods revealed that the pattern of chromosome behavior during yeast meiosis precisely parallels the same pattern in mammalian gametogenesis (spermatogenesis or oogenesis). A unique gene required for this germ line chromosome behavior was molecularly cloned from yeast and shown at the DNA sequence level to be evolutionarily conserved in higher organisms. This gene has been shown to be absolutely required for chromosome synapsis, faithful segregation, and subsequent viability in the germ line but to be completely dispensable for somatic growth of yeast. Immuno-cytological screening of sera from human patients with auto-immune or related diseases has revealed potentially similar patterns of recognition of nuclear structures in both yeast and mammalian cells. These sera are being further characterized to facilitate identification of conserved nuclear antigens which may serve as structural probes to study proper germ line chromosome behavior. Together, these observations indicate that the fundamental mechanisms of inheritance from one generation to the next are remarkably similar at the cellular and molecular levels in yeast and mammals. Thus, for the first time, there is good support for a non-animal (yeast) model of mammalian germ line chromosome behavior and inheritance.

Second, a rapid and efficient test system has been developed for yeast which allows a precise molecular characterization of the nature of mutation and genetic damage. An artificial yeast chromosome has been constructed that allows mutations in a target gene to be analyzed at the DNA sequence level. Using this system, the spectrum of spontaneous mutations has been determined; this is the largest database yet determined for any eukaryotic gene. Most significantly, it is the only test system in which one can measure genetic rearrangements caused by endogenous retroviruses. Several known mutagens and environmental effects have already been tested in this system, demonstrating its ease of use and its reliability. This system is being extended to examine the role of specific gene products in mutation avoidance and repair. Thus, a rapid and faithful test system for genetic damage in a eukaryote has been established.

A genetic system which had been developed for detecting copy number of centromere based plasmids in yeast is being utilized to determine tolerance of centromeres in cells of different ploidies in order to better understand mechanisms of chromosome segregation. The plasmid system is also being used to map DNA damage and repair in the centromere and to isolate damage-induced mutations. A protein has been identified in meiotic cells that appears to be involved in recombination, in that it enables exchange of DNA strands. The activity is lacking in the rad50 and rad52 mutants, whose products are required for double-strand break repair and meiotic recombination. A plasmid system has been developed for addressing the importance of DNA double-strand breaks as signals for the induction of repair systems and recombination.

In Drosophila, the ends of chromosomal terminal deficiencies that had been recovered earlier are being characterized molecularly. These chromosome ends are not completely stable; they lose DNA sequences at a rate of about 75 base pairs per generation. One unstable chromosome end has been cloned and the DNA sequenced. One DNA strand is rich in Guanine and Thymine with runs of consecutive Thymines, but the sequence does not have a regular pattern. Four stable lines have been identified. The newly stable chromosome ends are longer than the progenitor unstable chromosome. Additional telomeres of both the stable and unstable lines are being cloned to identify consensus DNA sequences that are necessary for chromosome stability.

Two regions of the X chromosome of Drosophila, one containing the mei-41 gene and one containing mei-9 have been cloned. Work is progressing on the molecular characterization of these genes. A nuclease gene on chromosome 2, genetically controlled by mei-41, has been identified and cloned based on similarities with a nuclease gene from yeast.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 21012-07 CGTB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Organ and Species Differences in Chemical Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Langenbach	Microbiologist	CGTB	NIEHS
Others:	K. Rudo	Biologist	CGTB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The ability of human and rodent tissues to metabolize known or suspected chemical carcinogens is being investigated. The metabolic profiles and genetic toxicities of the chemicals with human tissue activation are then being compared to the results from rodent tissues. Human and rodent liver and kidney cell metabolism of the model carcinogens, benzo(a)pyrene and acetylaminofluorene, have been studied. For human liver, nine individual tissue specimens have been investigated and for acetylaminofluorene eight of the nine human samples were more active metabolizers than the rat hepatocytes. The interindividual variation in the overall human metabolism was about 3-fold, although variation in individual metabolites was as high as 35-fold. The ability of human hepatocytes to conjugate these hydroxylated products with sulfate or glucuronic acid was also greater than in rat hepatocytes and the human interindividual variation to conjugate was about 8-fold. For benzo(a)pyrene, the differences in total metabolism between human hepatocytes and rat hepatocytes were less. Studies with kidney tissues have also indicated that human cells are more active than rat kidney cells in producing acetylaminofluorene metabolites; but kidney cells from both species are less active than hepatocytes. Again, about a 3-fold interindividual variation in human kidney metabolism was observed. Again with benzo(a)pyrene, differences in total metabolism between human and rat kidney cells were less than for acetylaminofluorene. The findings indicate that the extent of interindividual variations can vary with the chemical being studied. Furthermore, differences between human and rodent metabolism of chemical carcinogens can also vary with the chemical class, and understanding these species differences will be necessary in the extrapolation of rodent carcinogenesis data to humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21013-07-CGTB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Analysis of Gene Toxic/Carcinogenic Events in Mammalian Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. R. Boone	Microbiologist	CGTB	NIEHS
Others:	R. W. Tennant	Supervisory Microbiologist	CGTB	NIEHS
	K. Borroto Esoda	Biologist	CGTB	NIEHS
	C. L. Innes	Microbiologist	CGTB	NIEHS
	C. K. Heitman	IRTA Fellow	CGTB	NIEHS

COOPERATING UNITS *(if any)*

Wen K. Yang, Biology Division, Oak Ridge National Laboratory

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.6	1.6	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During the past year we have continued our studies of the regulation of retrotransposition/retrovirus integration. Our studies have focused on three areas. First, we have completed the development of a retrovirus packaging/vector system in which integration is restricted in mouse cells expressing the Fv-1^{b} allele but not the Fv-1^{n} allele. This is a unique system with which to study the mechanism by which the mouse Fv-1 gene product inhibits retrovirus DNA synthesis/maturation or integration into chromosomal DNA. Vector/packaging systems with different Fv-1 allele sensitivities are under construction. Secondly, we have analyzed the viral determinant for Fv-1^{b} tropism. Our evidence suggests that this is a property of a moderately abundant family (approximately 30 members) of endogenous MuLV-related proviral genomes. Thus, in mice carrying the Fv-1^{b} allele, selection against the Fv-1^{n} tropism determinant of endogenous ecotropic MuLV results in the acquisition of the B-tropic determinant from this more abundant class of endogenous sequences. Thirdly, in collaboration with Dr. Anton Jetten, LPP/NIEHS, we have begun a study of the regulation of the Fv-1 gene. Cell cultures derived from a common progenitor have been identified in which the Fv-1 gene is phenotypically silent in some clones and expressed in other clones. Preliminary evidence suggests that this will provide a model system for further analysis of the Fv-1 gene, including molecular cloning.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21016-07 CGTB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Enzymes Involved in DNA Repair and Meiosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M. A. Resnick Supv. Research Geneticist CGTB NIEHS

COOPERATING UNITS *(if any)*

Terry Chow, National Research Council, Canada

LAB/BRANCH

Cellular and Genetic Toxicology Branch

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INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several DNA metabolic processes are influenced or controlled by genes whose products function in DNA repair. The RAD52 gene of *Saccharomyces cerevisiae* plays a central role in many of the events in recombination and repair. It controls the repair of ionizing radiation-induced DNA double-strand breaks, radiation-induced spontaneous mitotic recombination, and recombination during meiosis, and is required for mutagenesis by some alkylating agents. Utilizing an antibody raised against a *Neurospora crassa* deoxyribonuclease, we had shown that an antigenically-related enzyme could be identified from yeast and that this enzyme is controlled by the RAD52 gene. The protein has been purified and is a single-strand exo- endonuclease and a double-strand exonuclease with MW = 72,000. It is not the product of the RAD52 gene. Using a lambda gtll vector expression library that contains genomic yeast DNA and the antibody as a probe, we have identified a segment of DNA that codes for cross-reacting material. A sequence from a yeast genomic library has been identifed that hybridizes with this segment. Introduction of this into a Rad+ strain leads to enhanced synthesis of cross-reacting material. The gene appears to be essential based on mutation of the cloned sequence and introduction into the genome. The transcription of this gene is under the control of the RAD52 gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21032-04 CGTB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Peroxidase Oxidation Systems in Mutation Assays

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William Caspary	Biochemist	CGTB	NIEHS
Others:	D. Daston	Biologist	CGTB	NIEHS

COOPERATING UNITS *(if any)*

Laboratory of Molecular Biophysics, NIEHS

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mechanisms of metabolism other than those mediated by the mixed function oxidases may be important in activating certain chemicals to their ultimate carcinogenic form. Prostagladin H synthetase is being used to activate compounds in the mouse mammalian cell forward mutation assay. Initial experiments showed hydrogen peroxide to be a good substrate for this enzyme. However, mutagenicity results are confounded by the interaction of hydrogen peroxide with sodium pyruvate, a media component in this assay. Other substrates are also being examined. Minimal treatment conditions and optional concentrations of purified enyzme have been determined. The possible mechanisms responsible for the formation of mutagenic metabolites induced by various peroxidase enzyme systems are being investigated. Selective inhibitors of the peroxidase are used to aid in the elucidation of these mechanisms. Studies include the identification of metabolites induced by this activation system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21035-04 CGTB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural Analysis of Meiotic Chromosome Behavior in Yeast and the Mouse

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. N. Giroux	Senior Staff Fellow	CGTB	NIEHS
Others:	M. Dresser	NRC Biotechnology Associate	CGTB	NIEHS

COOPERATING UNITS *(if any)*

Montrose Moses, Duke University, Durham, NC

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Cellular and Genetic Toxicology Branch

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INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The focus of this project is to investigate at the molecular level the structural basis of meiotic chromosome metabolism and segregation in the yeast, Saccharomyces cerevisiae, and to compare it to that of the mouse and related mammalian species. Methods of isolation and identification by light and electron microscopy have been developed for meiosis-specific structures in yeast based on surface spreading techniques combined with immunofluorescence. Whole-mount preparations have been used to demonstrate well-preserved synaptonemal complexes in preparations of yeast meiotic cells, as visualized by both light and electron microscopy. These new methods demonstrate for the first time that meiotic chromosome behavior in yeast closely parallels that in higher eukaryotes. Chromatin condensation and decondensation proceed in step with chromosome pairing, synapsis, and desynapsis. In concert, these events produce the classical stages of leptotene, zygotene, pachytene, and diplotene, demonstrating the utility of yeast as a model system for analysis of chromosome structure and function. A combination of cytological and molecular cloning techniques has demonstrated that the SPO11 gene of yeast is required for chromosome pairing and/or synapsis during meiosis. In contrast, chromosome pairing and synapsis proceed apparently normally in a deletion mutant of the RAD52 gene of yeast. Antibodies which recognize the synaptonemal complex in yeast and the mouse are being screened for by these new methods in order to identify protein components of the synaptonemal complex. An antigen associated with paired yeast chromosomes during meiosis has been identified.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 21039-04 CGTB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Genetic Control of Sister Chromatid Exchange in Yeast

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. A. Resnick	Supv. Research Geneticist	CGTB	NIEHS
Others:	R. Graetzer	IPA	CGTB	NIEHS

COOPERATING UNITS (if any)

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Cellular and Genetic Toxicology Branch

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INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The mechanism of sister chromatid recombination is poorly understood at the genetic and molecular level. Since it may be important in repair, mutagenesis and gene amplification, we have initiated studies to characterize the process and investigate the genetic control in the yeast Saccharomyces cerevisiae. Sister chromatid recombination is measured by recombination between two fragments of the HIS3 gene which share a 300 base pair region of homology. They are arranged in such a way that recombination will generate a complete gene. We have shown that several of the genes involved in DNA repair are required for the levels observed in wild type cells. Mutations in genes that enhance or depress homologous chromosome recombination have similar functions on the sister chromatid recombination process. DNA damaging agents can considerably enhance the process although the cells are resistant to x-ray induced exchange. In order to determine more accurately the levels of spontaneous sister chromatid recombination, we have developed procedures for greatly reducing the background levels. This is allowing small increases to be determined more accurately.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 21045-06 CGTB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of SPO11, a Gene Required for the Early Events of Meiosis in Yeast

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. N. Giroux	Senior Staff Fellow	CGTB	NIEHS
Others:	H. F. Tiano	Biologist	CGTB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Cellular and Genetic Toxicology Branch

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INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	0.6	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to identify and analyze the cellular functions which are required specifically for meiosis in the yeast, *Saccharomyces cerevisiae*. In particular, we are focusing on the analysis of the *SPO11* gene of yeast which is required for recombination and proper chromosome segregation during meiosis. A general system has been developed to isolate specific genes of yeast for which mutants are available. Using this system, the *SPO11* gene was physically isolated; this represents the first molecular cloning of a meiosis specific gene from any organism. The DNA sequence of the *SPO11* gene has been determined and a candidate polypeptide coding sequence of 398 amino acids has been identified and confirmed by hybrid gene fusions. This sequence predicts a strongly basic amino terminal domain. An in vitro engineered gene disruption has been used to demonstrate that the *SPO11* gene is essential for meiosis but is not required for vegetative growth or normal progression through the cell cycle. The cloned *SPO11* gene has been shown to be expressed only in meiotic cells. Thus, mutation in a single gene is sufficient to disrupt meiotic differentiation and proper chromosome behavior, giving rise to mostly inviable or grossly aneuploid products. Specifically, the *SPO11* gene is required for the assembly of the synaptonemal complex. The *SPO11* gene product is being expressed by recombinant DNA methods in *E. coli* to facilitate its biomedical characterization.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 21048-05 CGTB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of a Molecular System to Study Mutagenesis in Yeast

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. N. Giroux	Senior Staff Fellow	CGTB	NIEHS

COOPERATING UNITS *(if any)*

Bernard Kunz, Department of Microbiology, University of Manitoba, Canada

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The focus of this project is to investigate the mechanisms whereby genetic information is transmitted to progeny somatic cells with fidelity: how mutagenesis occurs, and what mechanisms the cell employs to avoid mutation. Using a combination of classical genetic and recombinant DNA techniques, we have constructed a model system to examine the molecular basis of mutagenesis in the yeast, *Saccharomyces cerevisiae*. Using this system, the spontaneous mutation rate in the target *SUP4-o* tRNA suppressor gene has been determined to be 2.7 X 10^{-7} events per cell division. The distribution (or spectrum) of mutations occurring spontaneously in the target gene has been determined and demonstrates that all types of single base substitutions as well as deletions may be detected reliably in this system. The *SUP4-o* system is being developed as a rapid genetic test for the induction of all types of mutation occurring within a eukaryotic gene which will also allow determination of the mutagenic specificities of agents giving positive responses. As a test of induced mutagenesis, we have characterized mutations induced by U.V. irradiation of yeast cells harboring the assay plasmid. U.V. induced all types of base substitutions occur at sites of adjacent pyrimidines, suggesting that they were targeted by U.V. photolesions. Hotspots for U.V. mutagenesis were detected in the target gene whereas no hotspot for spontaneous mutation has been observed. This work is being extended to an examinatiion of the spectrum of spontaneous mutation in strains which are mutant for genes required for mutation avoidance and/or repair. The first such mutant to be examined is the *rem1* hypermutator of yeast.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 21049-06 CGTB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
DNA Synthesis and Metabolism During Meiosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. A. Resnick	Supv. Research Geneticist	CGTB	NIEHS
Others:	A. Sugino	Visiting Scientist	LGM	NIEHS
	J. Westmoreland	Biologist	CGTB	NIEHS

COOPERATING UNITS *(If any)*

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Unique DNA metabolic activities have been implicated during meiosis and following exposure of mitotic cells to DNA damaging agents. We have characterized both the DNA and DNA metabolic enzymes at various times in meiosis in wild type and repair-deficient cells of yeast. No changes in the single-strand or double-strand size of chromsomal DNA are detected at any time during meiosis, while changes are observed in various mutants. Recombination is an important process in repair and in recombinaton. We are investigating proteins that might be involved in both processes. Previously we had shown that a RAD52 controlled nuclease increases nearly 10-fold, implicating it in meiotic recombination. We have purified a protein from cells that are undergoing meiosis that is able to carry out a strand exchange reaction. This reaction which involves the displacement of one of two strands from a duplex by another homologous single-strand DNA molecule is generally considered to be one of the basic steps in recombination that take place within cells. The protein has a MW = 38,000 and does not have ATPase activity nor is ATP required for the reaction. The appearance of the protein requires the RAD50 gene product and is meiosis specific. Strains that are homozygous for mating type (and therefore do not undergo meiosis) do not accumulate this protein during meiosis. The role of the RAD52 gene is currently being examined since it appears to have control function. This is being done by domain mapping, i.e., using different rad52 mutants. The importance of chromosome pairing is also being examined using strains which undergo meiosis as haploids.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 21051-05 CGTB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Cytogenetic Analysis of Mutagen-Sensitive Mutants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	James M. Mason	Geneticist	CGTB	NIEHS
Others:	Masayoshi Watada	Visiting Fellow	CGTB	NIEHS

COOPERATING UNITS (if any)

University of California, Davis
National Research Council, Canada

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.7	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mutants defective in DNA repair have been collected in Drosophila melanogaster and characterized cytogenetically in order to gain a basic understanding of the genetic control of sensitivity to mutagenic agents. Most of our recent work has centered on the mutants for the mei-9 and mei-41 genes. These mutants are sensitive to a wide variety of mutagenic agents, defective in excision and post replication repair respectively, and meiotic recombination, and have fragile chromosomes. The mei-41 gene is a hot spot for EMS and P-element insertion mutagenesis and shows a high frequency of interallelic meiotic recombination, suggesting that the gene is relatively large. To confirm this hypothesis and to better understand the structure and regulation of genes controling DNA repair, these two genes have been cloned and are being characterized molecularly. The mei-41 gene controls the activity of a specific DNA endo- exonuclease that is antigenically related to endo- exonucleases from yeast and Neurospora. A DNA sequence that codes for the nuclease in yeast has been hybridized in situ to Drosophila salivary gland chromosomes. A single site of hybridization has been identified in region 56D on chromosome 2; mei-41, however, is located in region 14C on the X chromosome. We are currently cloning the gene using the yeast sequence as a probe.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21052-06 CGTB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Metabolism of Xenobiotics to Mutagens by Non-Hepatic Enzyme Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Errol Zeiger	Supervisory Microbiologist	CGTB	NIEHS
Others:	Dennis Pagano	Microbiologist	CGTB	NIEHS
	Thomas Eling	Head, Prostaglandin Group	LPFT	NIEHS
	Thomas Petry	Staff Fellow	LPFT	NIEHS

COOPERATING UNITS *(if any)*

Laboratory of Pulmonary Function and Toxicology, NIEHS

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS: 0.3	PROFESSIONAL: 0.1	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Job completed September 30, 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21053-05 CGTB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic Control of Mutation in Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: James M. Mason Geneticist CGTB NIEHS

COOPERATING UNITS *(if any)*

University of California, Irvine

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.3	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is designed to determine the relationship between DNA repair, chromosome structure and mutagenesis in Drosophila melanogaster. A mutation that increases the mutation frequency (a mutator) has been identified and characterized. This mutator greatly reduces the efficacy of a repair pathway for x-ray induced chromosome breaks, thereby allowing a previously undescribed repair pathway to be observed. By this newly identified repair pathway individual broken chromosome ends are "healed," allowing the recovery of terminal deletions. The distribution of chromosome breaks that can be healed has been examined at several levels. (1) The mutator acts only in the female germline, not in males or somatic cells. (2) Breaks can be recovered anywhere in the euchromatin. (3) The mutator increases the recovery of breaks in the heterochromatin, but the breaks are not recovered as terminal deletions. Rather, they are rearrangements similar to the rearrangements from wild-type females. Thus, while the mutator itself does not have differential effects on euchromatin and heterochromatin, the healing process may be specific to euchromatin. (4) The breakpoints of the terminal deletions are distributed randomly, suggesting that specific DNA sequences are not required for the healing process to occur. (5) DNA sequences are being lost from the deficient chromosomes, suggesting that the neotelomeres on the broken ends are not as effective as the original telomeres and that the process of replicating chromosome ends involves loss and replacement of DNA sequences. The terminal restriction fragments of several of these deletions have been cloned. We plan to sequence the DNA of each fragment in order to compare it with the sequence of known telomeres. We are also developing a rapid assay for the mutator to facilitate insertional mutagenesis and transposon tagging.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21054-05 CGTB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

DNA Damage and Repair in Centromeres of Yeast

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. A. Resnick	Supv. Research Geneticist	CGTB	NIEHS
Others:	J. Westmoreland	Biologist	CGTB	NIEHS

COOPERATING UNITS *(if any)*

Kerry Bloom, Associate Professor, University of North Carolina, Chapel Hill

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.1	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Chromosome segregation requires a functional spindle apparatus, microtubules, chromosomal attachment sites, and a centromere specific DNA sequence. Disruptions of any of these organelles can lead to chromosomal malsegregation and aneuploidy. We are addressing two aspects of the function of centromeres within yeast cells: 1) the ability of cells to modify the number of centromeres; and 2) the ability of cells to deal with damage in the centromere. We are developing a plasmid system which allows for the genetic detection of the number of centromere-containing plasmids within a cell. This is being done by including within a centromere plasmid the gene for copper resistance CUP1. Increases in plasmid number lead to increased resistance. We have observed that cells can tolerate at least 8 additional centromeres and that this does not disturb growth or the process of meiosis. This system will enable an analysis of the relationship of the spindle apparatus organization to centromere function. Because of the systems we have available for detecting aneuploidy, it will be possible to determine consequences of altered centromere number on genome stability with a high degree of detection ($<10^{-5}$). Cells containing a large number of centromere plasmids are being used to examine repair in the centromere DNA. While it was previously possible to characterize damage and repair in this chromosomal organelle, the system was not sufficiently sensitive to precisely map damage and determine repair in relation to structure. The presence of a large number of the same centromeres will allow far more quantitative approaches to these issues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21091-03 CGTB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effects of DNA Lesions on Untargeted DNA Metabolic Events

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. A. Resnick	Supv. Research Geneticist	CGTB	NIEHS
Others:	C. Bennett	Visiting Fellow	CGTB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The effects of specific DNA lesions on cellular metabolism are poorly understood. Double-strand breaks (DSBs) is one category of lesions that is of considerable concern. They can occur as primary events as a consequence of repair. In addition they may appear during normal DNA metabolic events involving recombination. DSBs are known to be inducers of recombination.

In order to understand the consequences of DSBs on cellular DNA and their role as inducers of recombination, a system has been designed in the yeast Saccharomyces cerevisiae to study the effect of defined DNA double-strand breaks in plasmids on recombination and repair at various chromosomal sites. In this system, a diploid yeast strain with heteroallelic markers on homologous chromosomes harbors two plasmids: a low copy CEN plasmid that carries a gene coding for the site-specific endonuclease HO under the control of the inducing promoter GAL, and a stable plasmid in which copy number can be varied that carries a site at which the HO endonuclease cuts. Since the chromosomal HO-cut sites are deleted in this strain, depression of HO should generate a defined multiple DSB only at HO-cut sites carried by the plasmids. Preliminary experiments indicate that the depression of the HO gene and subsequent appearance DSBs in plasmids causes induction of recombination in the chromosome. The mechanism of this trans-acting effect of DSBs is being investigated in terms of effects on growth, survival, mutation, and recombination and genetic control.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 21094-02 CGTB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mutagenesis by Free Radicals From Bisulfite and Thiols

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Errol Zeiger	Supervisory Microbiologist	CGTB	NIEHS
Others:	Dennis Pagano	Microbiologist	CGTB	NIEHS

COOPERATING UNITS *(if any)*

A.-A. Stark, Department of Biochemistry, Tel-Aviv University, Israel

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS: 0.7	PROFESSIONAL: 0.2	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mutagenicities of sodium bisulfite and glutathione, reported earlier, appear to be mediated through the formation of oxygen-containing free radicals. The mutagenicity of bisulfite to bacteria is related to its autoxidation rate in solution, and this value varies with pH, temperature, and presence of other substances in solution with sodium bisulfite. The autoxidation rate changes with pH and temperature (pH 7/37C > pH 7/27C > pH 5/37C > pH 5/27C) and the mutagenic response increases as the rate decreases. The data suggest that for mutagenicity to occur, unoxidized bisulfite must be available to be taken up by the cells. The addition of ethanol, mannitol, liver homogenate (S9), or oxoid broth enhanced the responses but did not change the directions of the trends. These data suggest a role for sulfur and oxygen-centered radicals in the toxicity and mutagenicity of bisulfite.

Glutathione is mutagenic to bacteria via its autoxidation which leads to the formation of hydrogen peroxide, which is a bacterial mutagen. The Salmonella strains that are most responsive to glutathione mutagenicity are also the most responsive to hydrogen peroxide. Other thiols (cysteine, penicillamine, cysteine ethyl ester, and cysteinylglycine) were mutagenic without exogenous metabolic activation and their mutagenicity was a function of the pH of the medium and the pKa of the thiol group. This supports the hypothesis that the spontaneous oxidation of these thiols, with the eventual formation of hydrogen peroxide, requires the initial formation of a thiolate anion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21101-02 CGTB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mutant Selection Due to Differential Toxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William Caspary	Biochemist	CGTB	NIEHS
Others:	D. Daston	Biologist	CGTB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.5	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Job completed September 30, 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 21103-02 CGTB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluations of Genetic Toxicity Test Results

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Errol Zeiger	Supervisory Microbiologist	CGTB	NIEHS
Others:	Beth Anderson	Biologist	CGTB	NIEHS
	Walter Piegorsch	Mathematical Statistician	SBB	NIEHS

COOPERATING UNITS *(if any)*
Barry Margolin, Dept. of Biostatistics, UNC, Chapel Hill, NC

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A database of results from a number of short-term assays has been created using chemicals tested by the CGTB. A subsection of this database contains chemicals tested for carcinogenicity by the NCI and the NTP. This database allows the evaluation of each short-term assay with respect to its ability to predict carcinogenesis or other short-term assay results. It also permits studies of the individual assays with respect to inter- and intra-laboratory reproducibility, and the effects of protocol changes on test responses.

As a followup on an evaluation of the ability of four short-term tests (Salmonella mutagenicity; L5178Y mouse lymphoma cell mutagenicity; in vitro chromosome aberrations in CHO cells; and in vitro sister chromatid exchanges in CHO cells) to predict carcinogenicity using 73 chemicals, an additional 40 chemicals are being examined. These results will be used to determine if the results obtained with the original 73 chemicals were representative of the database as a whole. In addition to examining the qualitative predictivity of the assays, a study has been initiated to examine the predictivity of the assays as a function of the potency of the short-term test and carcinogenicity test responses.

More than 300 coded chemicals have been tested for mutagenicity in Salmonella in more than one laboratory, or at different times in the same laboratory. The inter- and intra-laboratory reproducibility of the assay is being examined, and differences in qualitative responses will be evaluated with respect to possible differences in test protocols. These analyses will lead to an assessment of the ability of short-term genetic toxicity assays to predict rodent carcinogenicity, and to the design of test protocols and strategies for the deployment of short-term tests.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21106-01 CGTB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

In situ Protocols for Mammalian Cell Mutagenesis Assays

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	William Caspary	Biochemist	CGTB	NIEHS
Others:	D. Daston	Biologist	CGTB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.5	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An in situ protocol for mammalian cell mutagenesis assays, in which cells are fixed during the expression and selection phases, was developed. It allows for the calculation of the mutation frequency, the proportion of new mutations in a population of cells, and rather than the mutant fraction, the proportion of mutants in a population of cells. The mutant fraction can give misleading assessments of the mutagenic activity of chemicals when a large number of mutants grow more slowly than the rest of the population. This protocol permits the calculation of mutation rates and we anticipate that it will give a more accurate assessment of the mutagenic activity of chemcials than standard protocols.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES 21107-01 CGTB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mutagenicity Studies of Hydrogen Peroxide and Hydrogen Peroxide Generating Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Amal Abu-Shakra	Visiting Fellow	CGTB	NIEHS
	Errol Zeiger	Supervisory Microbiologist	CGTB	NIEHS
Others:	Dennis Pagano	Microbiologist	CGTB	NIEHS

COOPERATING UNITS *(if any)*

A.-A. Stark, Department of Biochemistry, Tel Aviv University, Tel Aviv, Israel

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.2	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hydrogen peroxide (H_2O_2) is an endogenous chemical that is toxic and had been reported to be weakly mutagenic. We have studied the mutagenicity of H_2O_2 in Salmonella and optimized the conditions for its detection. The newly-constructed strain, SB1106+ gives a higher response than the standard mutagenicity testing strains TA97, TA100, TA102, or TA104. The mutagenic response will be correlated with the levels of catalase in the cells. An HPLC procedure for examining the DNA damage induced by H_2O_2 is being developed. Standards have been obtained or synthesized for major known oxygen-induced damaged bases (thymidine glycol; 8-hydroxydeoxyguanosine; 5-hydroxymethyl-deoxyuridine), and the DNA extraction and purification procedures are being developed for the Salmonella cells.

These procedures will be used to identify the pattern of damaged DNA bases induced by H_2O_2. Chemicals believed to cause mutation or damage DNA through the formation of oxygen radicals will be examined to determine whether H_2O_2 is an active intermediate or whether a different radical is the active product(s). This will be done by comparing the pattern of DNA damage with that produced by H_2O_2. Among the chemicals that will be examined are glutathione, cysteine, and other thiols that appear to be mutagenic via H_2O_2 formation, and metal ion complexes which are believed to produce active oxygen species.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60102-10 CGTB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Testing of Chemicals of Interest in Salmonella

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Errol Zeiger	Supervisory Microbiologist	CGTB	NIEHS
Others:	Dennis Pagano	Microbiologist	CGTB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.1	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A number of chemicals of interest have been tested for mutagenicity using Salmonella typhimurium in a number of different protocols. The rat liver carcinogen methapyriline was previously reported to be nonmutagenic in Salmonella. However, in this study, it produced a low-level, reproducible, dose-related mutagenic response in strain TA1535. The same response was also seen in two other laboratories. The mutagenic response seen was similar to the response seen with phenobarbital, another rodent liver carcinogen generally considered nongenotoxic. Phenobarbital is being studied, along with a series of chemicals having similar structures to portions of the phenobarbital molecule, in an attempt to elucidate its mutagenic mechanism.

HC blue 1 and 2 are both direct-acting mutagens but only HC blue 1 is a rodent carcinogen. We obtained purified samples of HC blue 1 which have been reported to be nonmutagenic, but retain their carcinogenicity. A "cleaned-up" sample of the mutagenic HC blue 1 lost approximately 90% of its mutagenicity, but retained its carcinogenicity. Another carcinogenic sample that had been synthesized by a different process was not mutagenic.

The human carcinogen treosulphan had not been previously reported as tested in Salmonella. In order to fill this data gap, it was tested in strains TA100 and TA1535, with and without metabolic activation. It was mutagenic in both strains under both activation conditions. Preliminary mutagenicity studies have been done on a crude preparation of red-tide (Ptychodiscus brevis) toxin. It was highly toxic to the Salmonella but not mutagenic. Additional studies have to be done to confirm the nonmutagenicity and to extend the study to other Salmonella strains and metabolic activation systems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60122-09 CGTB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Mechanisms of DNA Repair in Yeast and Their Role in Meiosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M. A. Resnick Supv. Research Geneticist CGTB NIEHS

COOPERATING UNITS *(if any)*
Dr. J. Nitiss, Harvard University, Cambridge, MA
Dr. J. C. Game, University of California, Berkeley, CA

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.1	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

DNA repair systems identified in mitotic cells of the yeast Saccharomyces cerevisiae are being examined for their protection of cells undergoing meiosis and the role of the corresponding genes in normal meiosis. The RAD50, RAD52 and RAD57 genes are essential in the repair of DNA double-strand breaks in mitotic cells. We have shown that they are also required for meiosis. Mutations abolish normal meiotic recombination; RAD50 acts early in meiosis. Rare single-strand interruptions (SSIs) are observed in rad52 and rad57 strains which appear to be related to recombination and these have been characterized. Based on genetic and biochemical changes, the order of gene function appears to be RAD50, RAD52, and RAD57. Given the important role RAD52 plays in repair and recombination, we have initiated studies to characterize its function in normal DNA metabolism and following treatment with DNA damaging agents. This is being done by "domain mapping" the functional regions of the RAD52 gene. Included among the processes affected by RAD52 are growth, recombination, mutagenesis, control of the essential yNUCR gene, control of strand exchange protein levels in meiosis, meiotic cellular viability, post replication repair, and DNA strand breaks in meisois. Mutations are being created in vitro and the altered gene is being transplaced into the genome. The consequences to the above gene functions are being examined. In addition, the role of the gene and mutants in relation to gene dosage is being examined using a system developed in this laboratory for isolating cell lines with different numbers of the gene.

CHEMICAL PATHOLOGY BRANCH

CHEMICAL PATHOLOGY BRANCH
Summary Statement

The Chemical Pathology Branch (CPB) functions to improve our understanding of the nature of the chemically induced lesions in rodents and to standardize our diagnosis, documentation and interpretation of these lesions. The CPB devotes a major share of its efforts to reviewing pathology data and histological diagnosis. The Branch is also responsible for the production, health status, and genetic integrity of the rodents used in the National Toxicology Program (NTP). In addition, the Branch conducts in-house studies and studies under contract, aimed at elucidating the nature and significance of pathological changes found in rodents. These efforts support the NTP mission to define the toxicity of chemicals of environmental concern.

The Tumor Pathology Section is responsible for the accuracy and validity of the pathology data derived from the two-year chronic studies. This section coordinates the histopathology quality assessment activities, organizes and conducts Pathology Working Group (PWG) reviews of the data from chronic studies, and evaluates and interprets the data for preparation of the NTP Technical Reports. During fiscal year (FY) 1988 the Tumor Pathology Section will have evaluated all aspects of the pathology data submitted to the NTP by the contract laboratories from 40 studies (Pathology Data Reviews). In addition, histopathology quality assessment will have been completed on 41 studies and PWG reviews will have been conducted on 33 two-year or lifetime studies.

Staff in the Tumor Pathology and Experimental Pathology Section (Clinical Pathology) are providing pathology support to the comprehensive toxicology studies of arsine gas. This collaborative effort involves staff from the intramural program of National Institute of Environmental Health Sciences (NIEHS) as well as the CPB. Arsine is widely used in a number of industrial processes and there is increasing use of arsine in the semiconductor industry for the production of gallium arsenide chips. A series of acute and subchronic inhalation studies in rats, mice, and hamsters that include histopathology and clinical pathology evaluations will be completed in FY 1987.

The Laboratory Animal Management Section provides genetically defined rodents of known microbial status for the NTP toxicology and carcinogenesis testing program. Two rodent production facilities are currently under contract to produce F344 rats and B6C3F1 mice. The rodent production is being modulated to provide an adequate number of animals to all new study starts and to decrease any excess production. Two diagnostic laboratories are conducting disease monitoring of NTP rodent production colonies and the animals on chemical toxicity and carcinogenicity studies. Sentinel animals in the toxicity and carcinogenicity studies are being used to assess the health status of animals on study. This program was useful in control of diseases in animals on study and currently more than 90% of the studies are free of viral infections.

Quality of the NIH-07 diet for the chemical toxicology studies is controlled by establishing limits on contaminant concentrations. These limits are being achieved by monitoring each batch of ingredient used in the manufacture of NIH-07 diet and each lot of diet selected for the toxicology studies. Studies to optimize the diet and feeding procedures for rodents in chemical toxicology studies are in progress. Projects to improve the mouse feeder and to develop unique and permanent identification methods and devices for positive identification of rodents in toxicology studies are in progress.

The Toxicologic Pathology Section assures the accuracy and validity of the pathology data from prechronic toxicity studies in rats and mice. Results of the prechronic data are interpreted for preparation of the NTP Technical Reports. Through the process of the PWG review, treatment-related lesions are confirmed and characterized for each sex and species. Based upon a combination of the "no-effect" level, severity and type of treatment-related morphologic changes, and other study data including mortality, clinical signs, body/organ weights, etc., dosage recommendations are given for each sex and species evaluated. During FY 1988, 48 prechronic studies were reviewed by the Toxicologic Pathology Section. Staff in the Toxicologic Pathology Section provide pathology support for additional in-house toxicologic studies performed by the Division of Toxicology Research and Testing (DTRT) at NIEHS. Pathology for special prechronic studies of theophylline, NPPD, vitamin E, and the evaluation of a microencapsulation technique will be completed by our staff in FY 1988.

Staff in the Clinical Pathology Laboratory are providing hematology and bone marrow cytology support of immunotoxicity studies on a variety of anti-AIDS drugs which have been under evaluation for the past year. This effort will continue for at least the next 12 months. These studies, which are being conducted in rats and mice, are designed to test for the toxic side effects of potential candidates for therapy of human AIDS patients. In addition, the Clinical Pathology Laboratory will continue to provide hematology and clinical chemistry support for scientists in the Intramural Division of NIEHS as well as for staff members in the DTRT and the CPB. During the past year the laboratory has supported studies involving arsine, dichlorvos, butoxyethanol, complex mixtures as part of the Superfund effort, and use of the mononuclear cell leukemia transplantation model to study the effects of the glycol ethers.

The Experimental Pathology section conducts collaborative research investigations in molecular biology, the role of cell proliferation in genesis of cancer, exploring the use of animal models to study roles of initiation and promotion in chemical carcinogenesis, morphometric analysis of chemically induced lesions, and use of magnetic resonance imaging to follow the progression and regression of chemically induced lesions in rodents. Most of these interrelated approaches are applied to tissues obtained from ongoing NTP toxicity and carcinogenicity studies as well as to tissues generated under contract using animal model systems. In the past clear differences in patterns of oncogene activation have been documented in spontaneous versus chemically induced rodent tumors. This work is being extended to determine the effect of different carcinogens on the activation of oncogenes, the role of oncogenes in the progression from hyperplastic to malignant lesions, and to characterize molecular lesions at the genomic level in chemically induced tumors in different strains and crosses of rodents. The purpose of these investigations is to characterize the underpinnings of the carcinogenic process and to determine if the processes operative in experimental rodent carcinogenesis as well as in carcinogenesis testing are applicable to human risk assessment. Investigations are currently underway to determine the role of chemically induced cell proliferation in genesis of lung and liver tumors associated with treatment by non-genotoxic carcinogens such as ethyl acrylate and methylene chloride. Both the cell proliferation measurements and the morphometric studies employ latest methods of computer assisted image analysis. Magnetic resonance imaging studies continue to show great promise and practical application in toxicity studies. We have recently demonstrated the progression and regression of toxic tubular necrosis in the kidneys of rats treated with mecuric chloride.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21111-01 CPB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Stability and Tissue Reaction of an Implantable Identification Device

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. N. Rao	D.V.M., Ph.D.	CPB	NIEHS
Others:	H. L. Amyx	D.V.M., BS	CMB	NIEHS
	J. Edmondson	Biologist	CPB	NIEHS

COOPERATING UNITS *(if any)*

Comparative Medicine Branch, Division of Intramural Research

LAB/BRANCH

Chemical Pathology Branch

SECTION

Laboratory Animal Management

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, N.C. 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.1	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Carcinogenicity studies require positive identification of test animals. Due to unreliability of ear notches and tags and inability to tattoo pigmented rodents, it is necessary to investigate more dependable and esthetically acceptable identification methods that can be read directly or by electronic means. The purpose of this study is to determine the stability, readability and tissue reaction of a microchip glass sealed device when implanted in the subcutaneous tissue of B6C3F1 mice for two years. Seventy B3C3F1 mice/sex were anesthetized, implanted and housed individually in polycarbonate cages. The devices were read by a radio frequency scanner weekly and palpated at monthly intervals. Ten mice/sex were necropsied at 3 months and this will be repeated at 15 months with the remaining animals to be evaluated at 24 months. Two of the 140 devices were lost and 1 failed by day 10. Devices were palpable and appear to be fixed at one location with no inflammation or palpable masses at 8 months. At the 3 month necropsy, implants were encapsulated by thin fibrous tissue, and a clear cyst was present around one implant. The transponder-detector system is working satisfactorily. However, alternately sized scanning/reading systems may be required based on various animal housing requirements. Procedures are in progress to convert the 10 digit random alpha-numeric identification of the implant to a more practical user number sequence.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21112-01 CPB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Growth Patterns of F344 Rats Fed NIH-07 and NTP-88 Diets

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. N. Rao	D.V.M., Ph.D.	CPB	NIEHS
Others:	J. Edmondson	Biologist	CPB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Chemical Pathology Branch

SECTION

Laboratory Animal Management

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, N.C. 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.1	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The maximum mean body weights of rats attained during the course of two-year studies increased by about 20% from 1975 to 1985. Higher body weights will lead to increases in the incidences of mammary tumors, pituitary tumors and possibly other tumors. Modification of diet and feeding procedures may slow the growth and lower the maximum body weight attained which in turn may decrease the incidences of spontaneous tumors. The purpose of this study is to determine the feasibility of a 15% protein diet (NTP-88) with restricted feeding from 4 p.m. to 8 a.m. in lowering the maximum body weights of rats in comparison with AD libitum feeding and 24% protein diet (NIH-07). Groups of 25M + 25F F344 rats housed 5/cage by sex are being fed NIH-07 or NTP-88 diet Ad libitum or 4 p.m. to 8 a.m. daily. Body weights and feed consumptions are being determined at one to two-week intervals. Hematology and clinical chemistry will be done at selected intervals and tissues will be collected for histology at the end of the study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21113-01 CPB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Myelotoxicity in Mice caused by Drinking Mixture of Groundwater Contaminants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. L. Hong	Biologist	CPB	NIEHS
Others:	G. A. Boorman	D.V.M., Ph.D., Chief	CPB	NIEHS
	R. S. H. Yang	Ph.D.	CTEB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Chemical Pathology Branch

SECTION

Tumor Pathology Section

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, N.C. 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Concern over the potential adverse health effects of chemically contaminated groundwater has existed for many years. In the present studies bone marrow parameters were monitored in female B6C3F1 mice exposed to a chemical mixture in drinking water for 17 days or up to 32 weeks. The mixture consisted of 25 common groundwater contaminants frequently found near toxic waste dumps, as determined by EPA surveys. The purpose of these experiments were to compare the hematopoisis among the different time courses of exposure and to determine whether residual damage could be demonstrated by subsequent radiations at a time when hematopoietic stem cells sustained injury or nearly recovered.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 21114-01 CPB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Pancreatic Nodule Transplantation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H. L. Hong	Biologist	CPB	NIEHS
Others:	G. A. Boorman	D.V.M., Ph.D., Chief	CPB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Chemical Pathology Branch

SECTION
Tumor Pathology Section

INSTITUTE AND LOCATION
NIEHS, Research Triangle Park, N.C. 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.15	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

DTRT has three cooperative agreements with universities to study the effects of corn oil administration on the exocrine pancreas of the rat. Part of their studies would require that rats be gavaged with corn oil for up to two years to produce hyperplasia and adenomas in F344 rats. Since most universities do not have the capability for repeated long term gavage in rodents, DTRT set up a contract with EG&G Mason to gavage 150 F344 rats with 10 ml. corn oil/kg 5 days a week for 100 weeks. In order to determine the number, size and transplantability of exocrine pancreas lesions in male F344 rats that have received corn oil by gavage for 75-100 weeks, the rats were necropsied, the pancreas weighed, nodules counted and portions of nodules transplanted to young male F344 recipients (one nodule/four recipients). If lesions are found, they will be transplanted into four recipients for up to ten lesions and at three months the recipients will be killed and examined. If growth is seen, one more three-month transplant. The ability to transplant part of a nodule and have histology on the rest may help validate our current classification scheme. The results indicate there is little or no growth on transplantation to the kidney capsule.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21115-01 CPB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effects of d-Limonene on α 2U-Globulin in Rat Kidney

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. L. Hong	Biologist	CPB	NIEHS
Others:	S. Eustis	Ph.D.	CPB	NIEHS
	G. A. Boorman	D.V.M., Ph.D., Chief	CPB	NIEHS
	C. W. Jameson	Ph.D.	CTEB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Chemical Pathology Branch

SECTION
Tumor Pathology Section

INSTITUTE AND LOCATION
NIEHS, Research Triangle Park, N.C. 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.09	0.09	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

d-Limonene is a natural component of a variety of foods and beverages and is found in many fruits, vegetables, meats, spices and other food items. Recently NTP conducted chronic two-year studies of d-Limonene in rats and mice and found there was clear evidence of carcinogenic activity for male F344 rats only as shown by increased incidences of tubular cell hyperplasia, adenomas and adenocarcinomas of the kidney. The response observed in male rats may be linked to specific renal pertubation of α 2U-globulin, unique to the male rat kidney. This study was performed to evaluate the hyaline droplet formation and the presence of α 2U-globulin in the kidney of male and female F344 rats after 14 days of gavage dosing at doses of 0, 75, 150, 300, 600 or 1200 mg/kg d-Limonene in corn oil. The α 2U-globulin was determined by the enzyme linked immunosorbent assay (ELISA) in the kidney homogenates. Total protein were measured in the same aliquots for α 2U-globulin by the Lowry method. We have confirmed that d-Limonene produced significant dose-related increase of α 2U-globulin only in male rats. These results suggest that d-Limonene-associated nephrotoxicity in male rats may be related to altered catabolism of α 2U-globulin, a low molecular weight protein synthesized by the liver under androgenic control.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21074-04 CPB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Glycol Ethers on Bone Marrow Parameters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. L. Hong	Biologist	CPB	NIEHS
Others:	G. A. Boorman	D.V.M., Ph.D., Chief	CPB	NIEHS
	J. Canipe	Biological Lab. Tech.	CPB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Chemical Pathology Branch

SECTION

Tumor Pathology Section

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, N.C. 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Ethylene glycol (EG) or ethylene glycol monomethyl ether (EGMME) was administered by gavage to both sexes of B6C3F1 mice for 4 consecutive days at total doses of 200, 400, and 1000 mg/kg body weight. Bone marrow parameters were examined on days 1, 5, and 14 after there final treatment. Exposure to EG produced hypocellularity and suppression of granulocyte-macrophage progenitor (CFU-C) colony formation in both sexes on days 1 and 5 postexposure. Values returned to normal by day 14 in the female mice but not in the males. Erythropoiesis, as measured by ^{59}Fe incorporation and quantitation of erythroid precursors in culture (CFU-E), revealed no effect in female mice and affected male mice at the high dose only. In contrast, EGMME exposure in female mice resulted in inhibition of erythropoiesis. There was also a pronounced effect on white blood cells with decreased peripheral counts, and decreases in the number of CFU-C's cultured from marrow cells. The effect of EGMME was also seen at the lower dose levels and was sustained through the 14-day evaluation period. In addition, EGMME caused a 20% decrease in testicular weight, which was shown microscopically to be a segmented degeneration of seminiferous tubules, an effect not found with EG. This study demonstrates that EGMME is more myelotoxic in mice than EG and that pancytopenia is more pronounced in males, while erythropoiesis is more affected in females. These results were accepted for publication in J. Environ. Path. Toxicol. Oncol., 1988 (In press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21080-04 CPB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Nuclear Magnetic Resonance Imaging Facility

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. R. Maronpot	Veterinary Pathologist	CPB	NIEHS
Others:	D. Dixon	Veterinary Pathologist	CPB	NIEHS
	G. A. Johnson	Radiologist	Dept. of Radiology	Duke Univ. Med. Center

COOPERATING UNITS *(if any)*

Duke University Medical Center
Durham, NC

LAB/BRANCH

Chemical Pathology Branch

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Magnetic resonance imaging (MRI) experiments are being conducted at Duke University Medical Center to explore the application of the technique during toxicologic studies. Animals (primarily rats) are anesthetized with a gaseous anesthetic (halothane), given complete and ventilation support, extensively monitored via electronic sensors to determine physiologic status and imaged for variable lengths of time (<1 hour to >4 hours) using a 30 cm bore, 2 Tesla MRI devise. Animals are imaged repeatedly (1 to 4 times a month) during a study. Investigations currently underway include imaging animals that are being treated to develop hepatic neoplasms to determine at what stage the neoplastic process can be detected and if the progression or regression of the tumors can be monitored. Additionally, studies are being conducted to explore the ability of MRI to detect acute renal damage in the rat. During 1988, a 7 Tesla magnatic imaging system will become operational and will provide resolution approximately 10 times greater than that currently available. This will permit the use of MRI in studies using mice in addition to rats. Several scientific articles have been published thus far. These relate to technical developments in small animal imaging, multiple imaging of the rat brain and imaging of the live, developing, chick embryo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21082-03 CPB

PERIOD COVERED

October 1, 1987 to September 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Residual Marrow Effect from Ethylene Glycol Mohomethyl Ether (EGMME) Exposure

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H. L. Hong	Biologist	CPB	NIEHS
Others:	G. A. Boorman	D.V.M., Ph.D., Chief	CPB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Chemical Pathology Branch

SECTION

Tumor Pathology Section

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, N.C. 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Ethylene glycol monomethyl ether (EGMME) has been reported to cause hematopoietic abnormalities in man. We have shown that mice exposed to EGMME postnatally have suppressed bone marrow cellularity and progenitor cells 8 weeks postexposure which returns to normal values by 16 weeks. Studies were designed to determine whether EGMME exposed mice that recovered had evidence of residual marrow stem cell injury. B6C3F1 mice were injected subcutaneously with EGMME on days 1-5 after birth at does of 0, 100, 200, and 400 mg/kg/day, allowed to recover, and stressed with 200 rads whole body irradiation at 15 and 21 weeks postexposure. Bone marrow functions were examined during the recovery period. Mice that had been exposed to EGMME were more sensitive to irradiation and recovery of marrow cellularity and progenitor cell numbers occurred more slowly than in unexposed controls. This indicates that EGMME can cause persistent residual damage of bone marrow progenitor cells in mice, an effect that would not be apparent with routine hematological techniques. These results were accepted for publication in Toxicol., 1988 (In press).

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21083-03 CPB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Myelotoxicity Induced in Female B6C3F1 Mice by Methyl Isocyanate Inhalation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. L. Hong	Biologist	CPB	NIEHS
Others:	G. A. Boorman	D.V.M., Ph.D., Chief	CPB	NIEHS
	J. Canipe	Biological Lab. Tech.	CPB	NIEHS
	J. Bucher	Ph.D.	CTEB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Chemical Pathology Branch

SECTION
Tumor Pathology Branch

INSTITUTE AND LOCATION
NIEHS, Research Triangle Park, N.C. 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.01	0.01	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The effects of a 4-day inhalation exposure (6 hr/day) to 0, 1 and 3 ppm methyl isocyanate (MIC) on bone marrow parameters in female mice were examined at 5, 8, 21 days and 1 year following exposure. The MIC exposure was associated with the bone marrow as evidenced by hypocellularity, suppression of pluripotent stem cells (CFU-S), granulocyte-macrophage progenitors (CFU-C) and erythroid precursors (CFU-E) in both dose groups. Hematopoietic parameters returned to normal by 21 days in the 1 ppm dose group, but not in the 3 ppm dose group. MIC is a highly reactive chemical that appears to exert its effect directly on the lining epithelium of the nasal cavity and major airways, and there was no histological evidence of a systemic effect. There was no significant effect on bone marrow cellularity and CFU-C in mice 1 year following acute exposure at the doses of 3 and 10 ppm for 2 hours. In conclusion, MIC exposure appears to cause acute cell death of lining epithelium of the nasal passages and major airways with transient alterations of bone marrow parameters that are likely related to the pulmonary injury either directly or secondary through the thymus. These results were publicated in Environ. Health Persp. 72, p. 143-148, 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21098-02 CPB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Adverse Effects of Lindane in B6C3F1 Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. L. Hong	Biologist	CPB	NIEHS
Others:	G. A. Boorman	D.V.M., Ph.D., Chief	CPB	NIEHS
	C. W. Jameson	Ph.D	CTEB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Chemical Pathology Branch

SECTION
Tumor Pathology Section

INSTITUTE AND LOCATION
NIEHS, Research Triangle Park, N.C. 27709

TOTAL MAN-YEARS: 0.25	PROFESSIONAL: 0.25	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Lindane (r-Hexachlorocyclohexane; r-Benzene hexachloride) is a popular insecticide. It is of interest to investigate the possible damaging action of this insecticide which is found in significant concentrations in everyday food (WHO, 1973). Male and female B6C3F1 mice are given Lindane at doses of 0, 10, 20 or 40 mg/kg daily for 3 consecutive days by gavage. Animals are killed on days 1, 2, 5 and 28 after the final treatment to study the histopathology, hematology and myelotoxicity of Lindane. We also examine the recovery of mice and the residual marrow effects following stress of radiation.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 21099-02 CPB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Hematopoietic Effects in Female B6C3F1 Mice Exposed to Arsine Gas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. L. Hong	Biologist	CPB	NIEHS
Others:	G. A. Boorman	D.V.M., Ph.D., Chief	CPB	NIEHS
	B. A. Fowler	Ph.D.	Tox. PRG.	U. Maryland

COOPERATING UNITS *(if any)*

LAB/BRANCH
Chemical Pathology Branch

SECTION
Tumor Pathology Section

INSTITUTE AND LOCATION
NIEHS, Research Triangle Park, N.C. 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.15	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Arsine gas is a potent hemolytic agent. Concern about semiconductor workers prompted an in-depth study of arsine at NIEHS to determine the hematopoietic effects of prolonged exposure to this gas. Female B6C3F1 mice were exposed by inhalation to 0, 0.5, 2.5, and 5 ppm arsine, 6 hr/day for 14 days. Body weights of exposed mice were comparable to controls, but a marked, dose-related splenomegaly was observed. Arsine exposure produced significant decreases in red blood cells, hematocrit and hemoglobin, with increases in white blood cells counts and the mean corpuscular volume of red blood cells. Furthermore, erythropoiesis as measured by quantitation of erythroid precursors in culture revealed significant reduction of CFU-E/femur cells for all treated groups and on day 3 postexposure and only at the 5 ppm dose group on 24 days postexposure. There was no alteration in bone marrow cellularity and less significant effect on granulocyte-macrophage progenitors. A 12-week study of arsine at 0, 0.025, 0.5 and 2.5 ppm (6 hr/day) by inhalation showed similar effects on hematopoiesis in mice. In addition, a depression of CFU-E was seen 3 weeks postexposure at 2.5 ppm group. In conclusion, arsine exposure at low doses produces a stress on the hematopoietic system characterized by a hemolytic anemia. These results are submitted (1988).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21100-02 CPB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Residual Hematopoietic Effect of Ochratoxin A(OCT A) in Mice Exposed to Irradiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. L. Hong	Biologist	CPB	NIEHS
Others:	G. A. Boorman	D.V.M., Ph.D., Chief	CPB	NIEHS
	C. W. Jameson	Ph.D.	CTEB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Chemical Pathology Branch

SECTION

Tumor Pathology Section

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, N.C. 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Ochratoxin A (OCT A) has the potential to cause myelotoxicity in addition to the well-know toxic effects on the liver and kidney. Experiments reported here were designed to determine whether mice would recover from the myelotoxic effects induced by OCT A injection and secondly whether mice previously injected to OCT A would be sensitive to radiation-induced myelotoxicity than vehicle controls. Six-week-old female B6C3F1 mice were injected intraperitoneally on alternate days over a week with a total dose of 0, 20 or 40 mg/kg of OCT A and bone marrow parameters monitored for up to 16 weeks. There was a suppression of marrow granulocyte-macrophage progenitors (CFU-C) in OCT A treated animals which returned to normal values by two weeks (20 mg/kg group) or by five weeks (40 mg/kg group) following the last treatment. Some of the OCT A treated mice were additionally irradiated with 200 rads whole body irradiation at 10 and 52 days following OCT A injections. Irradiation caused a significant reduction in CFU-C's in all mice but the effect were more pronounced in the mice that had received OCT A previously. The delayed recovery in bone marrow progenitors was also reflected in lower peripheral white blood counts after the second irradiation in 40 mg/kg OCT A treated mice as compared to the untreated irradiated controls. This indicated that residual bone marrow effect of OCT A makes the mice more sensitive to subsequent irradiation induced injury. These results were accepted for publication in Toxicol., 1988 (In press).

SYSTEMIC TOXICOLOGY BRANCH

SYSTEMIC TOXICOLOGY BRANCH
Summary Statement

Prediction of the potential for chemicals to adversely affect human health is best accomplished through extrapolation from toxicological data collected in laboratory animals. Groups within the Systemic Toxicology Branch (STB), in combination with those of other branches in the Division of Toxicology Research and Testing (DTRT), are designed to collect data to help characterize the toxicological profile of chemicals and also to collect data which help improve the methods for toxicological evaluation as well as better understand the mechanisms of toxicity of selected chemicals. The Systemic Toxicology Branch consists of five groups: Chemical Disposition, Developmental and Reproductive Toxicology, Immunotoxicology, Inhalation Toxicology, and Metals Toxicology. Each group is summarized below; for more details and specific accomplishments, consult the individual presentations on the following pages.

Chemical Disposition: Studies of chemical disposition within the NTP are conducted through outside contracts as well as an in-house program. The immediate objective of chemical disposition studies is to provide information on absorption, distribution, metabolism, and excretion of chemicals or chemical classes chosen for testing in NTP studies. This information is intended for use in design and interpretation of results of studies of toxicity and carcinogenicity of these chemicals. Long-range, but equally important, objectives of chemical disposition studies are to develop and publish data which will permit a better assessment of structure/activity relationships which influence chemical disposition or mechanisms of toxicity and provide basic information which will facilitate the extrapolation of laboratory data to humans. Through contracts or interagency agreements, the metabolism and disposition of a variety of chemicals has been investigated, including 4-vinylcyclohexene, 4-vinylcyclohexene diepoxide, theophylline, scopolamine, 1,1,1,-trichloroethane, β-bromo-β-nitrostyrene, 2,4-toluenediisocyanate, divinylbenzene, thiophene, 2-chloronitrobenzene, 4-chloronitrobenzene, benzene, butadiene, isoprene, vinylidine fluoride, and glycol ethers. Methods have also been evaluated, through contracts, by which human tissues can be used to study the metabolism of chemicals. Chemicals such as benzo(a)pyrene, benzidine, caffeine, and trichloroethylene have been investigated in human tissues to compare the metabolism to that in rat tissues. Metabolism in liver, kidney, lung, and adrenals has been evaluated. While the metabolic profile is generally similar, there were definite differences of quantitative and qualitative natures between the species.

Studies in-house have been designed to evaluate the disposition of such chemicals as polychlorinated dibenzofurans, TCDD, octachlorodibenzo-p-dioxin, polybrominated dibenzofurans, citral, tris(2-chloroethyl)phosphate, polyvinyl alcohol, ethyl carbamate, furan, 1,2,3-trichloropropane, and 2-butoxyethanol. Studies to evaluate the senescent changes in metabolism and their relationship to the expression of toxicity have continued. Also, studies have been conducted to evaluate the relationship between exposure to 2-butoxyethanol and resultant hematotoxicity in rats.

Developmental and Reproductive Toxicology: Studies of the potential of chemicals to adversely affect development and reproduction have been conducted through contracts as well as an in-house program of research. Through a contract and an interagency agreement, developmental toxicity studies were completed on n-hexane, tetrahydrofuran and acetone (all by the inhalation route), several glycol ethers, acrylamide and diethyl phthalate. Reproductive assessment studies by a continuous breeding protocol were initiated or completed on m- and p-nitrobenzoic acid, trichloropropane, sodium nitrite, methylphenidate, ethylene glycol monomethyl ether, boric acid, 4-vinylcyclohexene and carisoprodol; studies were also conducted on a chemical mixture (dump site contaminants), and the effect of dietary restriction was also evaluated. As a complement to our continuous breeding reproduction studies and to evaluate ovarian function as a target for toxic effects, we are examining the ovaries of female mice used in our continuous breeding studies through an interagency agreement with NCTR. This information should be useful guidance regarding methods of examining the ovaries and the correlation of structural changes with functional changes observed in continuous breeding studies.

Intramural projects continue in the areas of male fertility, postnatal toxicity, and teratology. In an effort to integrate animal model data with results in human semen evaluations as conducted through NIOSH, the rabbit has been investigated as a model for comparison to the human using ethylene dibromide as the test agent. Studies have been conducted on cimetidine, ranitidine and diphenhydrine to characterize the effects of these chemicals on lactation and postnatal development. Studies were begun to investigate the potential of chemicals to adversely affect the developing immune system of mice, using TCDD and DES as prototype chemicals to develop the methods for such investigations. The role of Sertoli cells in the expression of testicular toxicity continues to be investigated using cultured cells and test chemicals which are either known to affect testicular function or known not to affect testicular function. In conjunction with other work groups within the STB, reproductive and developmental studies were also conducted on some of the metals used in the semi-conductor industry, including arsine and gallium arsenide, in animals exposed by the inhalation route in the NIEHS inhalation facility.

Immunotoxicology: Effects of chemicals on the function of the immune system have been evaluated through contracts and through in-house research. Through contracts, the immunotoxicity of dideoxyadenosine, pentamidine (potential AIDS therapeutic agents), diaminotoluene and 4-chloro-o-phenylenediamine was investigated. Additional chemicals, benzothonium chloride, isobutyraldehyde, crotonaldehyde and isophorone diisocyanate were tested or are being tested for hypersensitivity. In-house studies were also designed to evaluate the potential effects on the immune system which could result from occupational, inadvertent, or therapeutic exposure to drugs, environmental chemicals, or biological materials. Studies were done to develop and utilize B-cell maturation as an *in vitro* model to sequentially examine the cellular and molecular events associated with chemical-induced immunotoxicity. Other model systems were evaluated which would allow assessment of neutrophil and alveolar macrophage function maturation potential, and ability to respond to physiological activation. Immunotoxicity associated with the tumorigenic process in mice neonatally exposed to diethylnitrosamine in combination with contaminated drinking water or chemicals with potential therapeutic use in AIDS patients was also investigated.

Inhalation Toxicology: This group conducts studies to evaluate the toxicity of chemicals where human exposure is primarily expected to be by the inhalation route. Research is focused on manifestations of toxicity at the levels of tissues, organs, and organ systems. The in-house program is integrated with that of Northrop Services, Inc., an on-site contractor with responsibility for conducting research and testing within the in-house inhalation facility. Major studies have been conducted this year to evaluate the toxicity and the mechanism of toxicity of inhaled arsine and gallium arsenide. These studies have been conducted in collaboration with other groups within the branch as well as elsewhere in DTRT. These studies are part of a series of studies to evaluate the toxicity of chemicals that are found in the semi-conductor industry. These studies focus on the adverse effects on hematopoiesis, the immune system, porphyrin metabolism, and reproduction and development. The data which are generated in these studies are extremely critical to NIOSH to facilitate their evaluation of the setting of standards for these chemicals. A chronic inhalation study on methylene chloride was started in female mice to investigate the effect of chronic exposure and age on metabolism of methylene chloride, the potential relationships between oncogene activation and tumorigenesis, and between cytotoxicity and tumorigenesis.

Metals Toxicology: Studies are conducted in-house through this group to characterize the toxicity of metals and the mechanisms of defense of the body against toxicity from metals. Studies were also conducted to evaluate the role of metallothionein and lead-binding proteins from rat, kidney and brain in regulation of the biological activity of lead. This group led the design and conduct of the inhalation toxicity studies to evaluate the toxicity of arsine and gallium arsenide.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 21003-08 STB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Disposition of Halogenated Dibenzofurans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Linda S. Birnbaum	Research Microbiologist	DTRT	NIEHS
Others:	David W. Brewster	IRTA Fellow	DTRT	NIEHS
	Yolanda Banks-Case	Biologist ("P" Appt)	DTRT	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.65	0.35	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Chlorinated dibenzo-p-dioxins (CDDs) and dibenzofurans (CDFs) are found worldwide as environmental pollutants. They have no known industrial use but are produced during the synthesis and breakdown of other chlorinated organic chemicals. They have been involved in several incidents of human poisoning. Brominated dioxins and furans are also suspected of posing an occupational and environmental hazard. Brominated napthalenes, a related group of compounds, were involved in an incident of human and animal poisoning in the past decade. The degree of halogenation correlates with the toxicity of these compounds, with those congeners possessing less than four halogens being relatively non-toxic. Halogenation in the four lateral positions is required for toxicity which then tends to decrease as the number of halogens increases. Metabolism of these chemicals is a detoxification process. Persistence can result in increasing body burdens upon repeated low dose exposures. While oral absorption is quite good (>70%), dermal absorption tends to be limited. However, as doses are decreased towards the levels which exist in environmental situations, as much as 40-50% of the applied dose may be absorbed through the skin. After a single application, absorption continued for several days at a slow rate. 2,3,7,8-TetraCDF (TCDF), which is metabolized relatively rapidly, was better absorbed than the related compounds, 2,3,4,7,8-pentaCDF, 1,2,3,7,8-pentaCDF, or 2,3,7,8-tetraCDD (TCDD). The disposition of 1,2,3,7,8-pentaCDF was examined in male rats. This isomer was distributed throughout the body (liver and adipose were the major tissue depots), and eliminated in the feces with a half-life of 2 days. Metabolism occurred prior to biliary excretion. These results are similar to those obtained with TCDF but are in contrast to those from studies with 2,3,4,7,8-pentaCDF and TCDD which are both poorly metabolized, and therefore persistent.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 21004-08 STB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Senescent Changes in Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Linda S. Birnbaum	Research Microbiologist	DTRT	NIEHS
Others:	Yolanda Banks	Biologist ("P" Appt.)	DTRT	NIEHS
	Timothy McMahon	IRTA Fellow	DTRT	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	0.6	0.6

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aging process can result in altered sensitivity to chemical toxicity. This can relate to either pharmacodynamic or pharmacokinetic factors. Current work examines the alterations that occur with advancing age in the processes of absorption, distribution, metabolism, and excretion of toxicants. Absorption through the GI tract does not change with age for compounds which are passively absorbed. Likewise, we have not observed changes with age (from 3 through 30 months) in the dermal absorption of either 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or 2,3,4,7,8-pentachlorodibenzofuran, two highly toxic environmental contaminants. Studies are in progress to determine whether the percutaneous absorption of a more readily metabolized isomer, 2,3,7,8-tetrachlorodibenzofuran (TCDF), will be affected with age.

While previous studies from this group have examined glucuronidation and glutathione conjugation changes with age, current efforts are focusing on glycine conjugation as an important detoxification route. Benzyl acetate, a model substrate, is hydrolyzed to benzyl alcohol, oxidized to benzoic acid, and then conjugated to form hippuric acid. There are no changes in this major route of metabolism, although there appears to be an increase with age in the levels of glutathione conjugation which occurs as a minor pathway. However, the rate of hydrolysis to benzyl alcohol appears to decline with age. The conjugation of salicylic acid, another substrate for the glycine transferase, has been reported to decline with age. In light of the lack of change in conjugation of benzoic acid, we are re-examining the disposition of salicylate in old rodents. It is possible that there is more than one enzyme involved in glycine conjugation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 21009-07 STB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Reproductive Effects in Males Exposed to Environmental Chemicals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert E. Chapin	Toxicologist	STB	NIEHS
Others:	J. J. Heindel	Expert	STB	NIEHS
	Jacqueline Williams	Visiting Fellow	STB	NIEHS
	Kimberley Treinen	Staff Fellow	STB	NIEHS

COOPERATING UNITS *(if any)*
Program Resources Branch, NIEHS
Comparative Medicine Branch, NIEHS
NIOSH

LAB/BRANCH
Systemic Toxicology Branch, DTRT

SECTION
Developmental & Reproductive Toxicology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.6	1.6	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Numerous environmental and industrial chemicals alter male reproductive function. One of the most sensitive tests in laboratory animals involves serial histopathology. We are coupling this approach with serial sperm analyses to evaluate how boric acid affects the male reproductive system in rats. In addition, we are evaluating the rabbit as a model for human reproductive toxicity. The rabbit is uniquely suited, as it is the only common non-primate lab species which shares with humans the ability to produce serial semen samples. Thus, this project is evaluating changes in rabbit seminal parameters (sperm endpoints, seminal biochemistry, pH, osmolality, etc.) after exposure to ethylene dibromide, a compound previously shown to affect human seminal endpoints. A critical addition to this project will be a test assessing the fertilizing capability of a constant number of spermatozoa from treated and control animals.

Significance: These studies continue our characterization of testicular lesion development, identify the most sensitive cell to boric acid, and evaluate the rabbit as a model for human reproductive hazard.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 21031-04 STB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Computer Simulation of Inhalation Exposures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	M. P. Moorman	Engineer	DTRT	NIEHS
Others:	R. A. Sloane	Biologist	DTRT	NIEHS
	R. S. Yang	Research Chemist	DTRT	NIEHS
	H. S. Kermani	Visiting Fellow	DTRT	NIEHS

COOPERATING UNITS *(if any)*
Northrop Services

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Inhalation Toxicology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.3	.2	.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A physiologically based pharmacokinetic model is being used to simulate the exposure of mice to methylene chloride (DCM) in a closed exposure system. Exposures performed in the closed exposure apparatus have been used to determine the rate of DCM disappearance from the test atmosphere as a function of concentration. This data is used in conjunction with the simulation to estimate whole body (global) metabolic rate constants for DCM. Measurements are being performed on animals taken from an ongoing chronic exposure at different time points to determine changes in these global rate constants as a function of both age and length of treatment. A refined model incorporating these changes will be used to provide estimates of the quantity of exposure compound and metabolites presented to target organs during the chronic exposure. The closed exposure system will be used in conjunction with the computer simulations as part of other inhalation studies to estimate the amount of exposure compound taken up by the test animals as a function of exposure concentration.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21033-04 STB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Disposition of Xenobiotics

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Linda S. Birnbaum	Research Microbiologist	DTRT	NIEHS
Others:	Usha Gundimeda	Visiting Fellow	DTRT	NIEHS
	Janet Diliberto	Biologist	DTRT	NIEHS

COOPERATING UNITS (If any)

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.35	0.35	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An understanding of pharmacokinetic factors can assist greatly in both dose-setting for toxicity studies and in the interpretation of the results. Selected chemicals on-test by the NTP are nominated for disposition studies. The absorption, both oral and dermal, distribution, metabolism, and excretion of these chemicals are studied in rats and other species as needed. The effect of dose on disposition is determined, as is the effect of the route of exposure. These studies help to predict the results obtained upon chronic exposure. Xenobiotics to be studied are radiolabeled with ^{14}C or ^{3}H by custom syntheses. Distribution and excretion are compared after iv, oral, and/or dermal exposures at several doses, the highest being 1/10th of the LD50. Disposition after an iv dose is examined at multiple time points after treatment. The excreta, expired air, and volatiles are analyzed for radioactivity which is resolved into parent compound and metabolites by organic solvent extraction and chromatography. Metabolites are then characterized by chemical and/or enzymatic means. Current work has focused on the disposition of citral ("oil of lemon"), a common flavoring and fragrance. We have characterized its absorption after both oral and dermal exposures. Because of its volatility, much of a dermal dose becomes unavailable for absorption. Some of the citral is oxidatively decarboxylated and metabolized to carbon dioxide. Most of the citral is eliminated in the urine. However, some of the citral is metabolized and enters the anabolic pathways of the organism. No citral can be detected in the blood within 5 minutes of an iv treatment. There are many metabolites of which the mono- and di- carboxylic acids of the two isomers which make up citral, geranial and neral, and their glucuronide and sulfate conjugates appear to be especially important.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21034-03 STB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Evolution of Metallothionein-like Proteins in Non-Mammalian Species

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: B.A. Fowler Research Biologist DTRT NIEHS

COOPERATING UNITS *(if any)*

C.F. Chignell and R. Hall, Laboratory of Molecular Biophysics; D. R. Winge, University of Utah; J.S. Garvey, Syracuse University; E. Gould, NMFS/NOAA

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Metals Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 21038-06 STB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Chemical Metabolism and Disposition

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	H. B. Matthews	Research Chemist	DTRT	NIEHS
OTHERS:	L. T. Burka	Research Chemist	DTRT	NIEHS

COOPERATING UNITS (*if any*)

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	0.4	1.7

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Chemical disposition studies are designed to provide both basic and applied knowledge of the fate and toxicity of selected chemicals in support of the National Toxicology Program. As such, these studies are designed to investigate both the effect of chemical structure and properties on acute and chronic toxicity and, whenever possible, investigate the effect of these factors on mechanisms of chemical toxicity and carcinogenicity. Recent studies in chemical disposition have addressed species-specific variation in the metabolism and clearance of ethyl carbamate and have provided data to document the significantly more rapid metabolism of this chemical by mice than rats. This data has further demonstrated that most studies of ethyl carbamate carcinogenicity have used doses which saturate metabolism and thus may not be optimum for extrapolation of animal data to predict human risks. Another study has addressed the fate of a preparation of polyvinyl alcohol designed for use with a spermicide. This work has demonstrated that absorption of polyvinyl alcohol from the gastrointestinal tract is negligible, but absorption may be significant when this compound is applied intravaginally. Further, the absorbed polyvinyl alcohol appears to be retained in the liver and other tissues and only slowly eliminated. Current work in this group is also addressing the species-dependent toxicity of tris(2-chloroethyl) phosphate observed in chronic studies of this compound. This work has demonstrated that this compound is readily absorbed from the gastrointestinal tract, but is not excreted prior to metabolism. The metabolites, once formed, are rapidly excreted in urine. This work has further demonstrated that metabolism may be saturated by doses which result in chronic toxicity. Therefore, it appears that the toxicity of tris (2-chloroethyl) phosphate may be attributed to parent compound.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21046-05 STB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Postnatal Toxicology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lori A. Dostal	Senior Staff Fellow	STB	NIEHS
Others:	B. A. Schwetz	Supervisory Pharmacologist	STB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch, DTRT

SECTION

Developmental & Reproductive Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies were conducted to characterize the toxicity of drugs and chemicals to neonates relative to adults, and to evaluate the role of lactation in the induction of neonatal toxicity. To evaluate the rat as a model for human milk excretion of chemicals and drugs, several different types of drugs were evaluated for their excretion into rat milk, and the data were compared with that previously obtained in humans. Using gas chromatography and high pressure liquid chromatography, the amounts of several drugs in rat milk and plasma were determined, as were the effects on the quantity and composition of the milk of the mothers. Water soluble, basic drugs diphenhydramine, cimetidine, and ranitidine were studied. The antihistamine diphenhydramine was present in rat milk in concentrations higher than those in plasma. There were no effects on milk composition and milk synthesis. Cimetidine was found in very high concentrations in milk relative to plasma, and studies were performed to investigate the mechanism of secretion of high amounts of cimetidine into milk. These studies examined protein binding in milk and serum, active transport into mammary gland and kidney slices in vitro, and the kinetics of elimination of cimetidine from plasma and milk. The effects of exposure to ranitidine during lactation on milk production and composition, as well as on the suckling pups, were also determined. The amount of ranitidine present in rat milk after oral dosing was greater than the concentrations in plasma and was similar to results reported in humans. Studies were initiated to determine the amount of nickel secreted into the milk after subcutaneous dosing and the subsequent effects on the suckling offspring. Future studies will examine the excretion of water soluble acidic drugs, nonionizable compounds, and other metals into rat milk.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 21070-05 STB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
TCDD Teratogenicity: Modulation in Mixtures

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Linda S. Birnbaum	Research Microbiologist	DTRT	NIEHS
Others:	Richard Morrissey	Biologist	DTRT	NIEHS
	Martha Harris	Biologist	DTRT	NIEHS
	Eric Haskins	Bio. Lab. Technician	DTRT	NIEHS
	Janet Allen	Bio. Lab. Technician	DTRT	NIEHS
	Laurie Couture	Biologist	DTRT	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	0.2	1.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) has been shown to be highly teratogenic in multiple species, causing cleft palate (CP) and hydronephrosis (HN) in the mouse at doses with no overt maternal or fetal toxicity. This response can be used as a method of determining whether compounds are dioxin-like or not. The interaction of several polychlorinated dibenzofurans (PCDFs) and certain polychlorinated biphenyls (PCBs) with TCDD are additive. However, the nonplanar PCB isomer, 2,4,5,2',4',5'-hexachlorobiphenyl (HCB), can antagonize the induction of CP by TCDD, only in a very narrow window - 8000-33000 times as much HCB as TCDD. We have not observed any inhibition of HN. In fact, HCB at 1000mg/kg may induce HN by itself. Treatment of pregnant mice with TCDD and retinoic acid (RA) causes an increase in the incidence of CP. However, RA does not affect the TCDD-induced incidence of HN, nor does TCDD alter the incidence of RA-induced limb bud abnormalities. RA interacts additively with TCDD when treatment is on gestation day (gd) 10, however, the interaction is synergistic on gd 12. Hydrocortisone (HC) can also enhance the incidence of CP when given in combination with TCDD. Thus, TCDD can interact with endogenous-type growth factors to cause fetal abnormalities. TCDD is a developmental toxin in rats. The closely related compound, 2,3,4,7,8-pentachlorodibenzofuran, failed to cause HN in Fischer rats when administered on gd 8, 10, or 12 at doses as high as 300 µg/kg where extensive maternal and fetal toxicity and CP did occur. Whether this is related to maternal toxicity or is specific to TCDD remains to be determined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21075-05 STB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Xenobiotic Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. T. Burka	Research Chemist	DTRT	NIEHS
Others:	H. B. Matthews	Research Chemist	DTRT	NIEHS
	P. Srinivas	Visiting Fellow	DTRT	NIEHS
	Nik Mahmood	IRTA Fellow	DTRT	NIEHS
	Diane Overstreet	Chemist	DTRT	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.1	2.1	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Knowledge of the metabolism and disposition of a xenobiotic is often critical to an appreciation of the toxic effect(s) of the compound. Further, the fidelity of extrapolation of results from animal testing to possible human health effects is greatly enhanced by the knowledge of metabolic pathways. Investigation of the mechanistic aspects of metabolic processes allows greater understanding of how metabolism of a xenobiotic might lead either to detoxification or to a reactive species with greater toxicity. As more is learned about mechanisms of metabolism, more accurate prediction of the possible metabolic pathways for new compounds should be possible. As part of a continuing effort to study xenobiotic metabolism, this group has investigated the metabolism of 1,2,3-trichloropropane, furan and tetrachlorodibenzofuran. The metabolism and disposition of 14C-1,2,3-trichloropropane have been investigated in male and female rats. The highest concentrations of radioactivity are found in liver, forestomach and kidney after 24 hr. Two urinary metabolites, both from the mercapturic acid pathway, have been identified. The chemical reactivity of butenedialdehyde, the suspected reactive intermediate from furan metabolism, is being investigated. Reaction of the dialdehyde with cysteine-containing compounds results in formation of substituted furans. Evidence for this reaction sequence occurring in vivo is being investigated. The structure of the metabolites of TCDF, a highly toxic contaminant often found in PCB's, continues to be under investigation. Two possible metabolites which have hydroxyl groups in place of chlorines have been synthesized recently. The presence of these compounds in bile of rats treated with TCDF is under investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 21084-03 STB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Association of Chemically Induced Forestomach Cell Proliferation & Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Burhan I. Ghanayem	Sr. Staff Fellow	DTRT	NIEHS
Others:	H.B. Matthews	Research Chemist	DTRT	NIEHS
	Robert Maronpot	Pathologist	DTRT	NIEHS

COOPERATING UNITS *(if any)*

Chemical Pathology Branch, DTRT, NIEHS

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Chemically induced cancer is a major concern of the National Toxicology Program (NTP). The mechanisms by which cancer is induced following exposure to chemicals are diverse and not well understood. Further, there are very few good model or test systems for studies of chronic insult by chemicals. Present work by this group has focused on the induction of cancer in the forestomachs of experimental animals receiving oral doses of test chemicals. This work was designed to follow the development of the early stages of these lesions in an effort to characterize their development from early lesions to fullscale cancers. The purpose of this work has been to determine the relevance of these lesions to human exposure to chemicals and evaluate the suitability of this lesion as a short term test for chemical toxicity and carcinogenicity. Results of this work have shown that a series of chemicals which have been characterized as stomach carcinogens induce hyperplasia and hyperkeratosis on acute exposure at doses which induced cancer in chronic studies. These lesions were dose dependent and significantly increased over those observed in controls in every case in which a known forestomach carcinogen was studied. Further, structurally related chemicals which did not induce cancer in chronic studies did not induce similar lesions when administered at comparable doses. This work is being extended to further characterize the development of these lesions following exposure to additional chemicals and to more closely examine development of these lesions in the course of repeated dosing over a period of several months and to determine the effect of coadministration of other drugs and chemicals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21086-03 STB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
2-Butoxyethanol Hematotoxicity: Effects of Age and Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Burhan I. Ghanayem	Sr. Staff Fellow	DTRT	NIEHS
Others:	L.T. Burka	Research Chemist	DTRT	NIEHS
	H.B. Matthews	Research Chemist	DTRT	NIEHS
	R.R. Maronpot	Pathologist	DTRT	NIEHS

COOPERATING UNITS *(if any)*

Chemical Pathology Branch, DTRT, NIEHS

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This work has been completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21089-02 STB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of Action of Testicular Toxicants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Co-PI:	Jerrold J. Heindel	Expert	STB	NIEHS
Co-PI:	Robert E. Chapin	Toxicologist	STB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch, DTRT

SECTION

Developmental & Reproductive Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	0.6	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Various environmental and industrial chemicals can perturb male reproductive function. The objectives of these studies are to define subcellular target sites in testicular somatic cells in primary culture. For FY88, efforts have focused on effects of mono-2-ethylhexyl-phthalate, Δ9 tetrahydrocannabinol, and the active metabolite of tri-o-cresyl phosphate (TOCP), saligenin cyclic o-tolyl phosphate, on Sertoli cells in primary culture. Since TOCP needs to be metabolized to an active intermediate in vivo, and because the testis has more of this active metabolite than most other tissues in the body, studies have been initiated to evaluate the capability of Leydig cells to activate TOCP in vitro, and to investigate the relationship of this activation to the Sertoli cell response to the saligenin in vitro. Endpoints for these studies have included overall energy balance, intermediary metabolism control, and "throughput," enzyme activity, cytoskeletal distribution by immunostaining. The emphasis continues to be on the dose- and time-relationships between these endpoints.

Second messengers (cyclic AMP, calcium, and inositol trisphosphate) are important regulators of cellular function. We have recently initiated a series of studies to determine whether testicular toxicants exert some of their effects by altering these second messenger systems. Compounds for these studies are the same as above: MEHP and Δ 9 tetrahydrocannabinol.

The significance of these studies is that they have identified structures and processes within these somatic testicular cells which are vulnerable to toxicants. A greater knowledge of where and how compounds work will further our understanding of how the cells work, and could help avoid toxicity for novel compounds in the future.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21090-03 STB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanisms of Arsine Gas and Gallium Arsenide Toxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. P. Moorman	Engineer	DTRT	NIEHS
Others:	B. A. Schwetz	Supervisory Toxicologist	DTRT	NIEHS
	R. E. Morrissey	Toxicologist	DTRT	NIEHS
	G. J. Rosenthal	Biologist	DTRT	NIEHS
	G. A. Boorman	Veterinarian Pathologist	DTRT	NIEHS
	H. L. Hong	Research Biologist	DTRT	NIEHS
	P. C. Blair	Supervisory Biologist	DTRT	NIEHS

COOPERATING UNITS *(if any)*

Northrop Services

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Inhalation Toxicology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1.5	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies are being conducted to evaluate the acute and short-term toxicity of arsine gas. Fischer 344 rats, $B6C3F_1$ and C57BL/6 mice, and Syrian golden hamsters have been exposed to arsine gas at concentrations of 10 ppb to 50 ppm for periods ranging from .5 hr to 90 days. All groups exposed to a single 6 hr exposure of 25 ppm experienced 100% mortality, while those exposed to 5 ppm for as long as four weeks or 2.5 ppm for 13 weeks showed no overt signs of toxicity. A dose-dependent increase in spleen weights and a slight increase in liver weights was observed at necropsy. Microscopic examination of spleens from exposed rats showed sequestration of macrophages and increased erythropoiesis. Blood samples showed small decreases in packed cell volume and a marked increase in ALAD activity. Urine samples showed elevated levels of coproporphyrin and 7 and 8 carboxyl uroporphyrin species. The effects on both sexes and all three species were similar. The data suggests that alterations in the heme biosynthetic pathway may be used as early biological indicators of ongoing arsine toxicity. Single exposures to 25 ppm for .5 to 3 hours caused severe loss of circulating RBC's which were observed as early as 8 hours after the initiation of exposure. RBC counts began to recover four days after exposure but had not returned to control values after seven days.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21093-02 STB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of Dioxin Toxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Linda S. Birnbaum	Research Microbiologist	DTRT	NIEHS
Others:	Charles D. Hebert	Biologist ("P" Appt)	DTRT	NIEHS
	Barbara Abbott	IRTA Fellow	DTRT	NIEHS
	Sidney Stohs	IPA	DTRT	NIEHS
	Laurie Couture	Biologist	DTRT	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.2	2.0	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TCDD has a broad range of effects which are species and tissue specific. The relative dermal toxicity and promoting ability of two polychlorinated dibenzofurans (PCDFs), 2,3,4,7,8-pentaCDF (PeCDF) and 1,2,3,4,7,8-hexaCDF (HCDF) was examined using the hairless mouse. TCDD is approximately 2.5X and 6X as dermally toxic as PeCDF and HCDF which is less of a difference than would have been predicted based upon studies of acute toxicity. Both PCDFs were also potent skin tumor promoters. The effects of TCDD on enzyme induction and glucocorticoid receptors were compared in the liver and skin of haired and hairless mice. The TCDD effects were tissue specific. No effect of TCDD was detected on the epidermal growth factor receptor in the skin. Whether these effects are under the control of the Ah receptor is currently being examined using congenic mice. The role of growth factors in TCDD toxicity is currently being studied using human squamous carcinoma cell (SCC) lines which have been reported to differentiate and/or proliferate in response to TCDD. The effect of transforming growth factor (TGFβ) is also under investigation. TCDD causes cleft palate (CP)and hydronephrosis (HN) prenatally in mice. HN is due to hyperplasia of the ureteric epithelium as well as a decrease in density of the extracellular matrix proteins surrounding the developing glomeruli. The reversibility and functional implications of HN are under investigation. TCDD-and retinoic acid (RA)-induced CP is due to failure of fusion of the palatal shelves. However, the events at a cellular level are distinct, with TCDD causing the medial edge cells to become oral-like and RA inducing a nasal-like epithelium. Organ culture techniques are being used to further dissect out the events involved in palatal fusion and kidney development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21105-01 STB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Heat Shock Proteins in Testicular Somatic Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Co-PI:	Robert E. Chapin	Toxicologist	STB	NIEHS
Co-PI:	Randy L. Allen	Staff Fellow	LRDT	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch, DTRT

SECTION

Developmental & Reproductive Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cells exposed to elevated temperatures respond by synthesizing heat shock proteins (HSP). This same set of proteins is also synthesized in response to other stressors: heavy metal ions, anoxia, or some toxic agents. The expression of HSP is thought to represent an adaptive and protective response of cells under stress. This project has two objectives: 1) to determine the HSP response of testicular somatic cells (Sertoli and Leydig cells) to known inducers of HSP in other cell types; and 2) to evaluate HSP secretion after exposure in vitro to toxicants known to affect the testis in vivo. If the latter effort is successful, the secretion of HSP in the testis in vivo after insult will be characterized to evaluate HSP as markers of toxicant-induced testicular injury. Ultimately, this project investigates the possibility of using HSP as a set of in vivo markers which would indicate that the testis has been injured.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 21109-01 STB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of 2-Butoxyethanol Induced Hematotoxicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Burhan I. Ghanayem	Toxicologist	DTRT	NIEHS
Others:	L.T. Burka	Research Chemist	DTRT	NIEHS
	H.B. Matthews	Research Chemist	DTRT	NIEHS
	J.M. Sanders	Biologist	DTRT	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previous work done in this laboratory showed that acute exposure to 2-butoxyethanol (BE; ethylene glycol monobutyl ether) causes severe hemolytic anemia. The present investigations were conducted to elucidate the mechanisms of BE-induced hemolytic anemia. The kinetics of ^{14}C-BE metabolism and clearance were studied in control adult (3-4 mo) and old (12-13 mo) male F344/N rats and in rats treated with pyrazole or probenecid. These studies showed no difference in the half-life (t1/2) of BE in adult and old rats. However, t1/2, area under the curve (AUC), and maximum plasma concentration (MPC) of its major metabolite, butoxyacetic acid (BAA) were significantly greater in older rats. This difference was more pronounced at high doses. Pretreatment of rats with pyrazole resulted in a significant increase in the t1/2 and AUC of BE, but significantly decreased MPC of BAA. Probenecid had no effect on t1/2, AUC, or MPC of BE, but significantly increased the t1/2 and AUC of BAA. The mechanisms of BE-induced hematotoxicity were also investigated _in vitro_. Incubation of BE with blood from F344 male rats caused no hemolysis and resulted in no metabolic alterations of BE. In contrast, incubation of BAL or BAA caused time- and concentration-dependent swelling of RBCs followed by hemolysis, however, BAA was significantly more efficacious than BAL. These effects of BAA and BAL were associated with a parallel depletion of Blood ATP. Similar _in vitro_ studies indicated that BAA causes minimal swelling or hemolysis of human RBCs at concentrations several fold higher than what is required to cause complete hemolysis of rat RBCs. Further, BAA caused minimal or no effect on human blood ATP levels. Investigation of the Structure-hematotoxicity relation-ships of alkoxyacetic acids _in vitro_ revealed that maximum effect was obtained with an alkoxy chain of 4 carbons (butoxy) and declined with 6 carbons chain (methoxy<ethoxy<propoxy<butoxy>pentoxy). Similar relationships were found in regard to the effect of alkoxyacetic acids on blood ATP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 21110-01 STB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Developmental Immunotoxicity Studies of Inbred Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Richard E. Morrissey	Toxicologist	STB	NIEHS
Other:	P. Lindstrom	IRTA	STB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Systemic Toxicology Branch, DTRT

SECTION
Developmental & Reproductive Toxicology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Chemicals such as 2,3,7,8-tetrachlorodibenzodioxin (TCDD) adversely affect immunologic function in offspring following treatment of pregnant mice late in gestation. TCDD or diethylstilbesterol (DES), another immunotoxicant, are administered to pregnant C57BL/6 mice at various times during gestation to: 1) establish the sensitive period for induction of immunologic deficits and 2) identify the initial lesion. T and B lymphocytes, and subpopulations of these cells, are immunocytochemically stained to determine the effects of these chemicals on immune system development. Quantitation is by flow cytometry. Studies to explore the inter-relationships of developmental immunotoxicity with the induction of structural malformations, weight gain, and transplacental carcinogenesis are planned.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30044-12 STB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Toxicology of Environmental Chemicals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	B.A. Schwetz	Chief, STB	DTRT	NIEHS
OTHERS:	M. P. Moorman	Engineer	DTRT	NIEHS
	R. A. Sloane	Biologist	DTRT	NIEHS

COOPERATING UNITS *(If any)*

Northrop Services, Incorporated

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Inhalation Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.9	.5	.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rats, mice and hamsters have been exposed by inhalation to arsine gas. The collaboration of many different groups within the DTRT has made it possible to investigate a variety of toxicological endpoints including histological, reproductive, hematological, immunological, and biosynthetic effects. Pilot exposures to methylene chloride have been completed and a two-year chronic exposure is in progress as part of a study being conducted in collaboration with several other groups within the NIEHS. This study is designed to investigate cellular and molecular processes responsible for the induction of lung and liver tumors and to measure changes in pharmacokinetic parameters with age and treatment. The ability to accurately control and document the exposure environment continues to be enhanced by refinements in methods of data acquisition and management. A system has been designed and implemented to manage the acquisition of data from in-life observations, weighings, and necropsies. This data combined with the environmental documentation provides a readily accessible history for each animal in a study. Work is continuing on the detailed design of a new inhalation facility. The floor plan has been completed. The design of special services, such as furnishings, containment enclosures, and air handling, control, and filtration equipment is in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30106-14 STB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Effects of Environmental Pollutants on the Immune System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael I. Luster	Research Microbiologist	STB	NIEHS
Others:	M. Ackermann	Visiting Fellow	STB	NIEHS
	G. Rosenthal	Biologist	STB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Systemic Toxicology Branch, DTRT

SECTION
Immunotoxicology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.0	3.0	5.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Immunotoxicology Group studies the adverse effects on the immune system resulting from occupational, inadvertent, or therapeutic exposure to drugs, environmental chemicals, and biological materials. The ongoing objectives include efforts: (1) to evaluate and examine the influence of selected drugs or environmental chemicals on the immune response and relate alterations in immunological functions with general and specific organ toxicity; (2) when applicable, to examine potential mechanism of action; (3) to relate changes in immunological functions with altered host resistance following challenge with tumor cells or infectious agents; and (4) to refine and validate a panel of immune and host resistance procedures in order to better define immunotoxicity and correlate changes in immune function with altered host resistance. Studies were performed in the following areas: (a) Development and utilization of B cell maturation as an in vitro model to sequentially examine the cellular and molecular events associated with chemical-induced immunotoxicity. General methodology includes the use of flow cytometry as well as methods for examining second messenger, cellular proliferation, and cellular differentiation. Chemicals and drugs that have been examined include compounds that modulate arachidonic acid products, tetrachlorodibenzo-p-dioxin, polycyclics, pertussis toxin, and methotrexate. (b) Development of model systems which allow assessment of neutrophil and alveolar macrophage function, maturation potential, and ability to respond to physiological activation. Endpoints for these assays include production of soluble mediators, surface markers, and effector cell function. (c) Evaluation and examination of immunotoxicity associated with the tumorigenic process in mice neonatally administered a liver promotor (diethylnitrosamine), in mice exposed to a contaminated drinking water sample, and in mice exposed to antinucleosides used in the treatment of AIDS (i.e., AZT, DDC, and DDA) or other AIDS therapeutics (e.g., α-interferon, AL721).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70200-14 STB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Mechanisms for Regulating for Intracellular Bioavailability of Metals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. A. Fowler	Research Biologist	DTRT	NIEHS
Others:	D. Gilg	Visiting Fellow	DTRT	NIEHS
	P. Goering	NRSA Postdoctoral Fellow	DTRT	NIEHS
	G. DuVal	NRSA Postdoctoral Fellow	DTRT	NIEHS

COOPERATING UNITS *(if any)*

C.F. Chignell, Laboratory of Molecular Biophysics

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Metals Toxicology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
This project is completed.

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